

Figure 1

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Figure 2a

Figure 2b

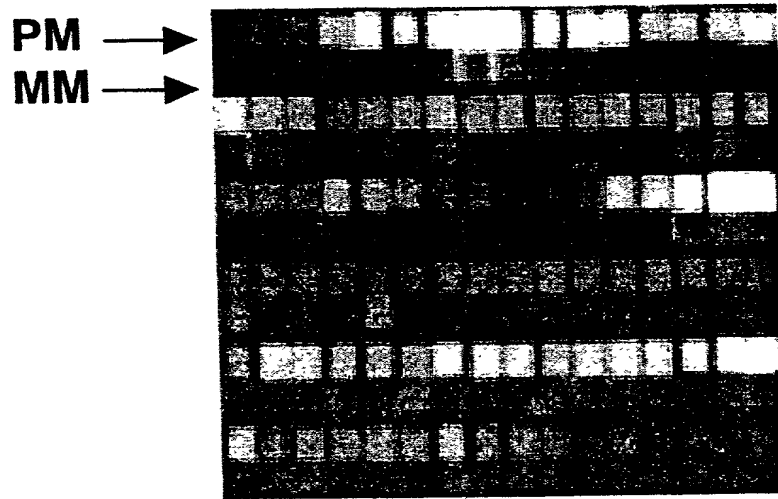
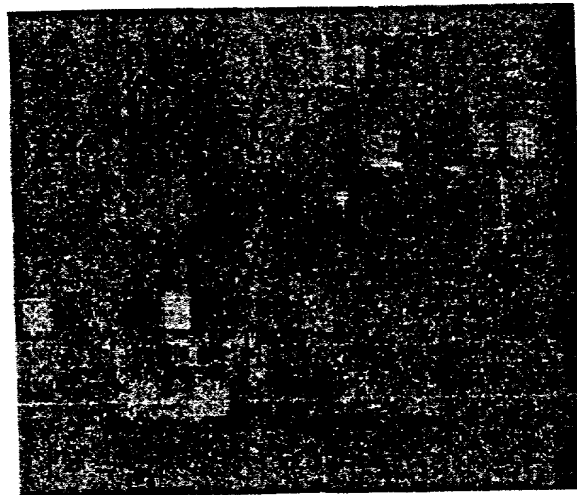


Figure 2c



Hybridization Signal vs Target Concentration

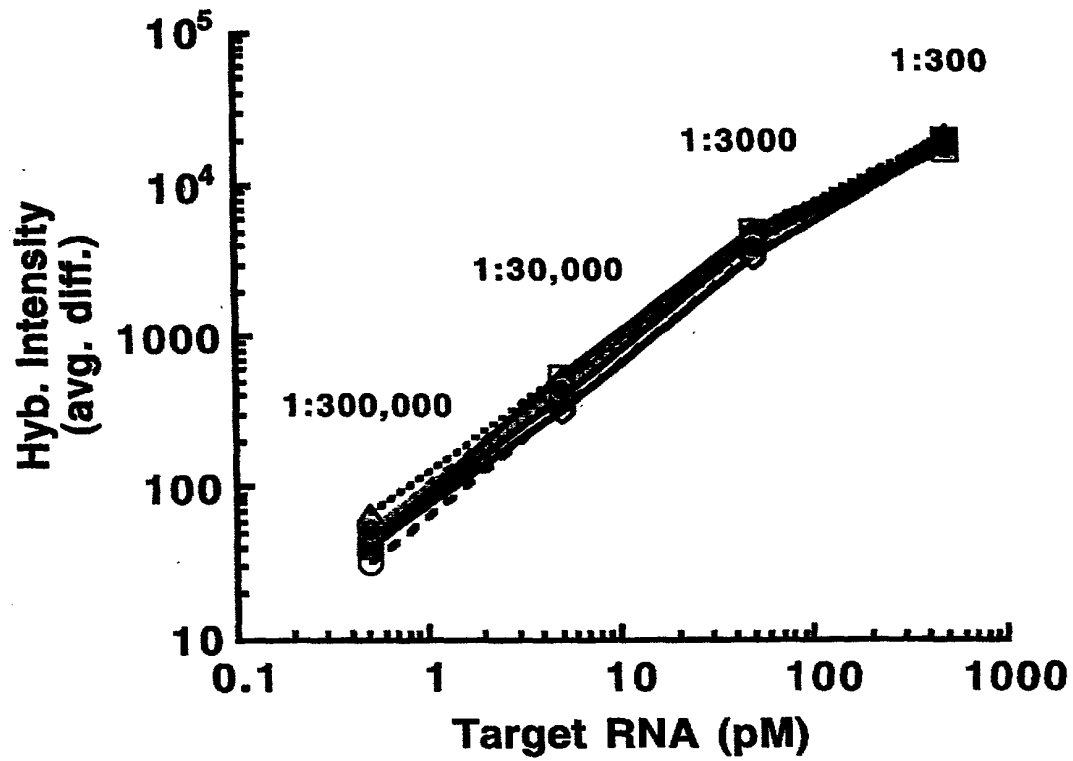


Figure 3

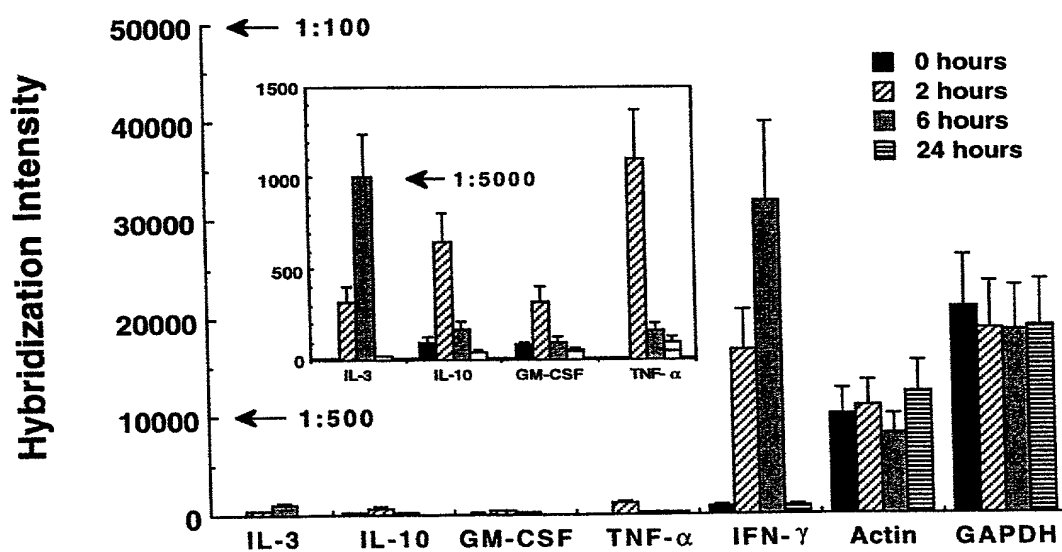


Figure 4

6/47

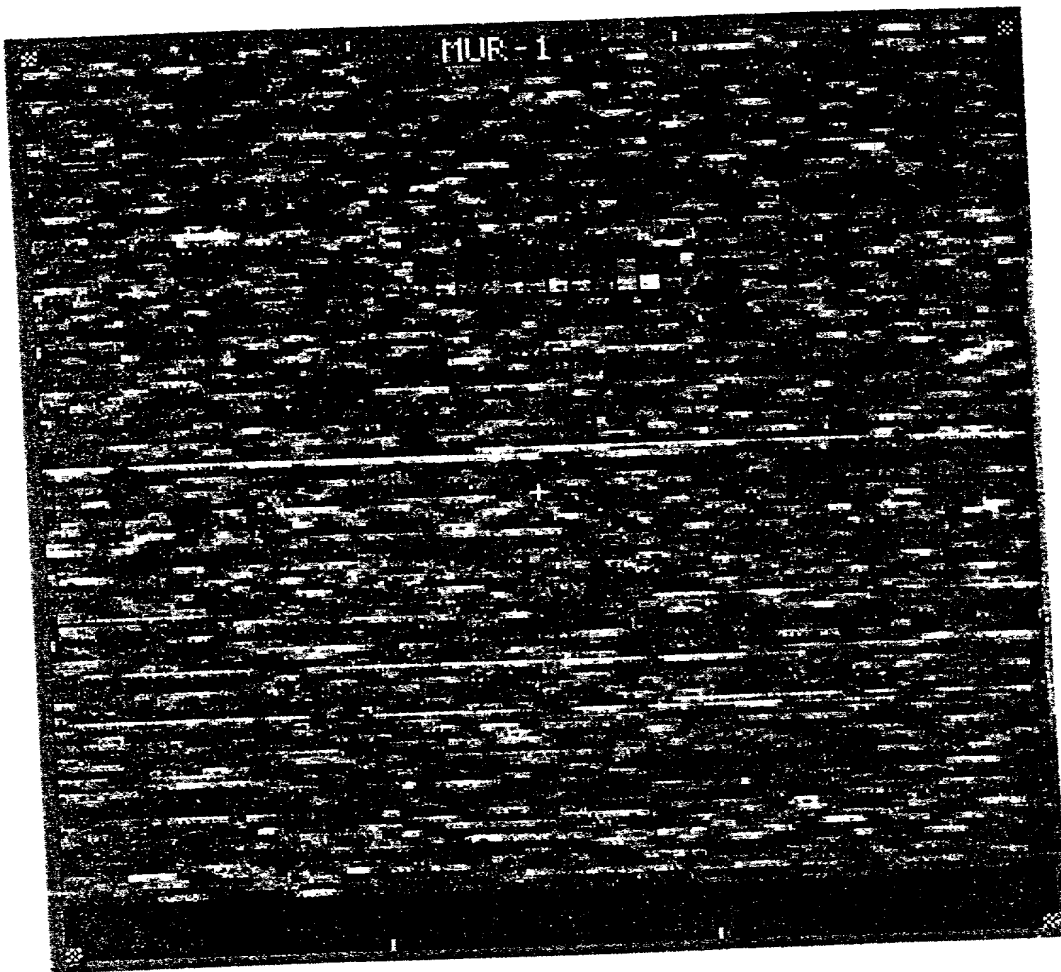


Figure 5

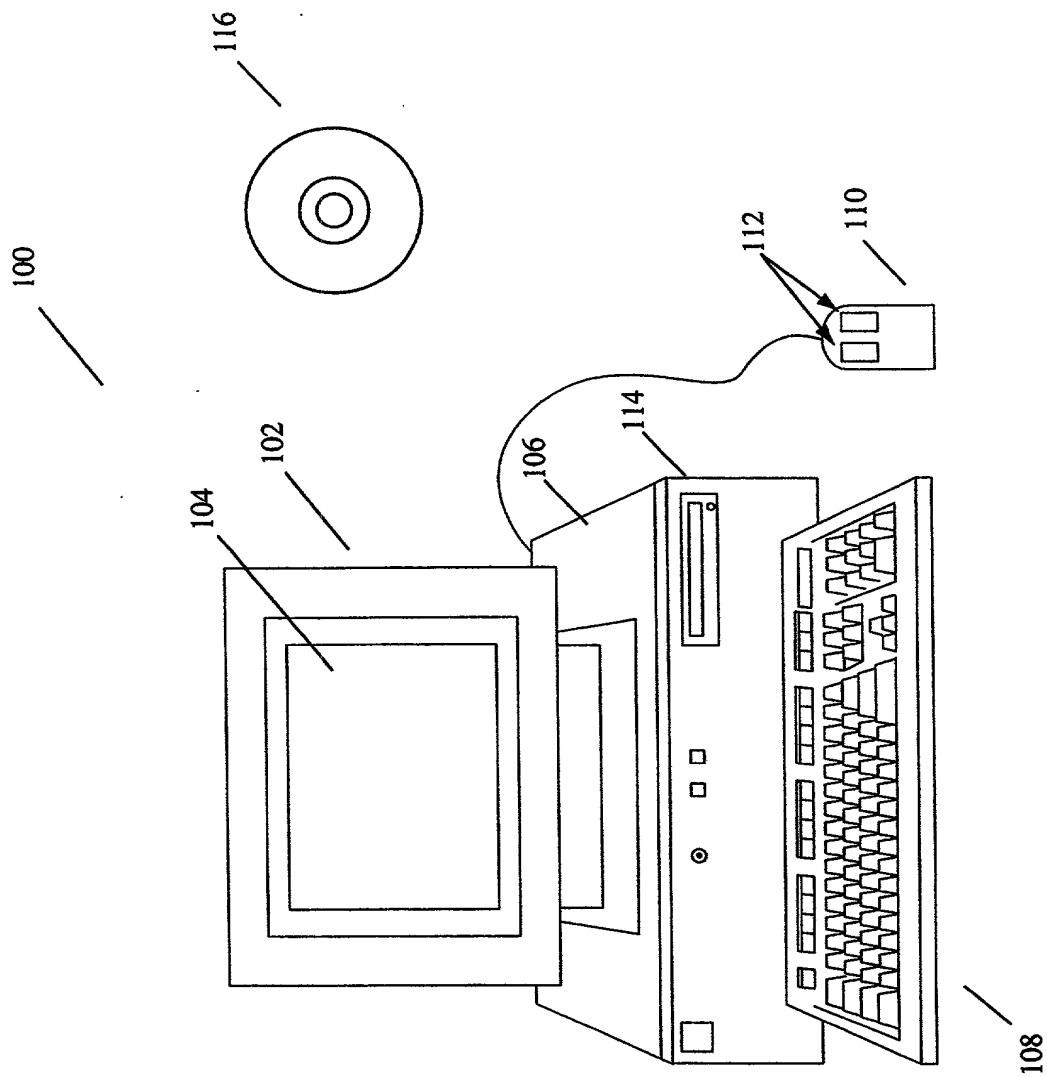


Figure 6

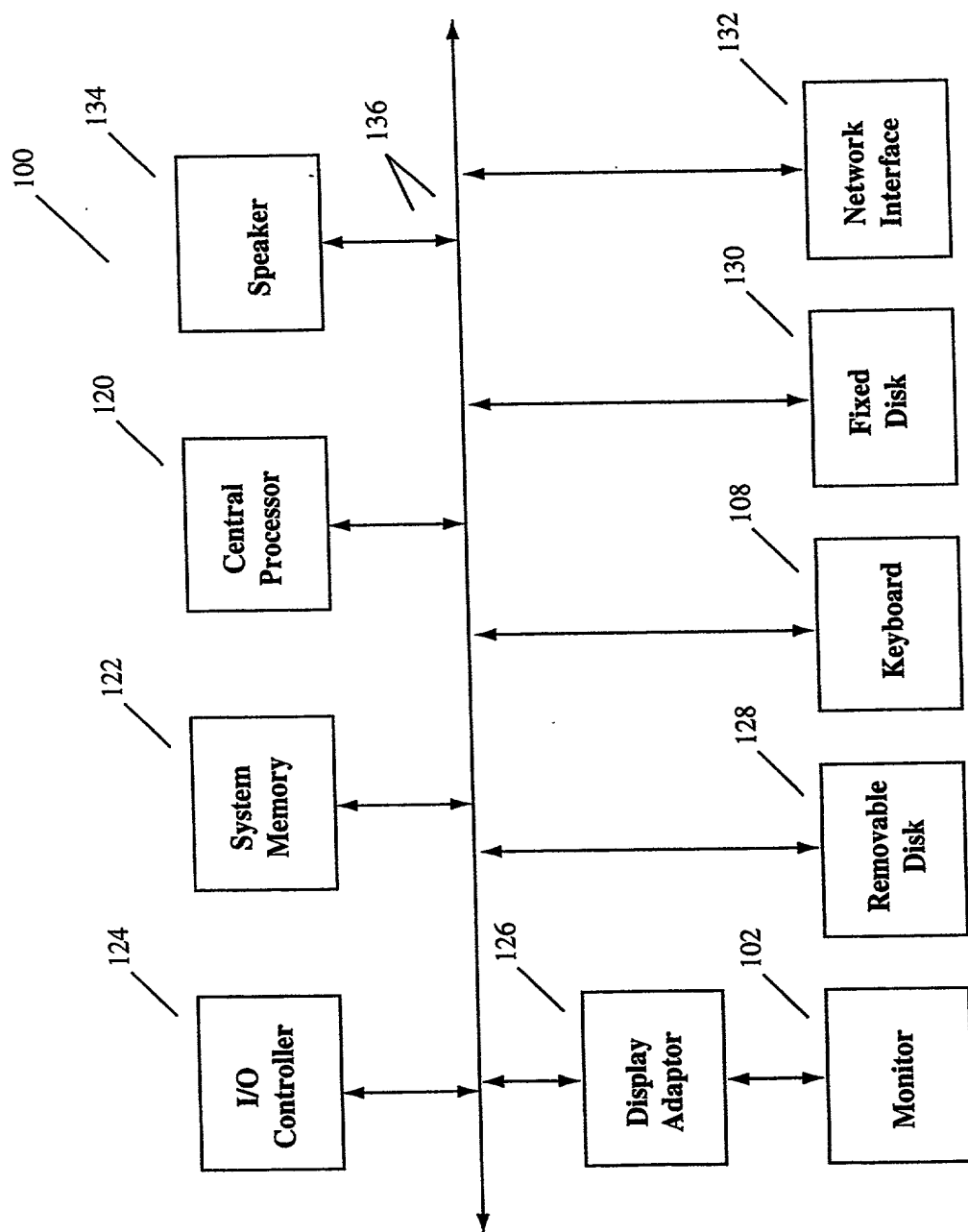


Figure 7

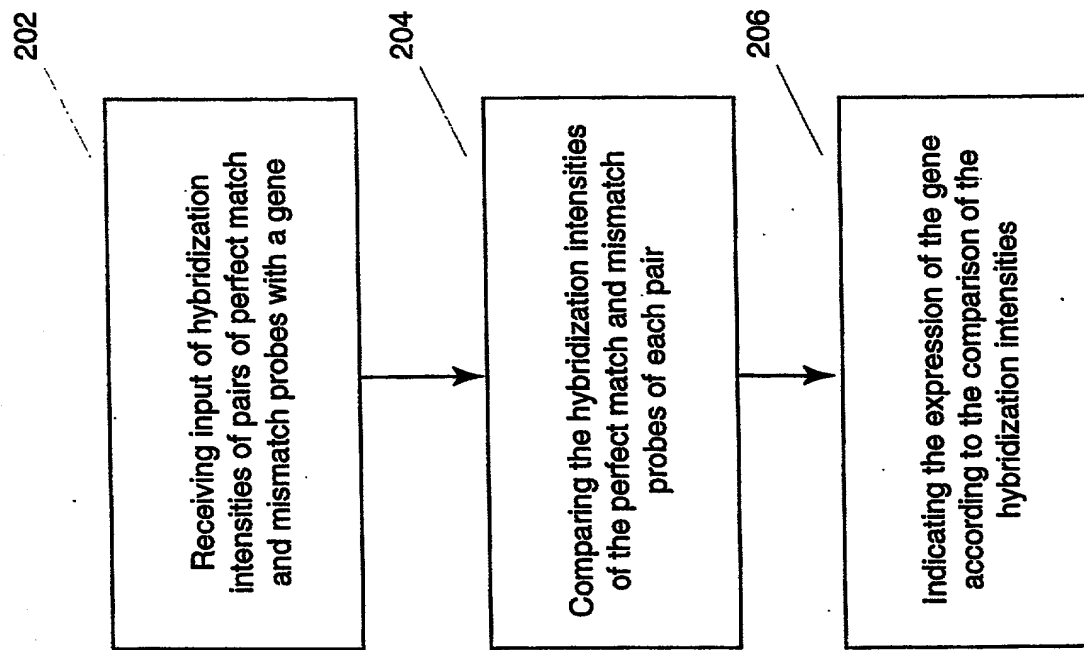


Figure 8

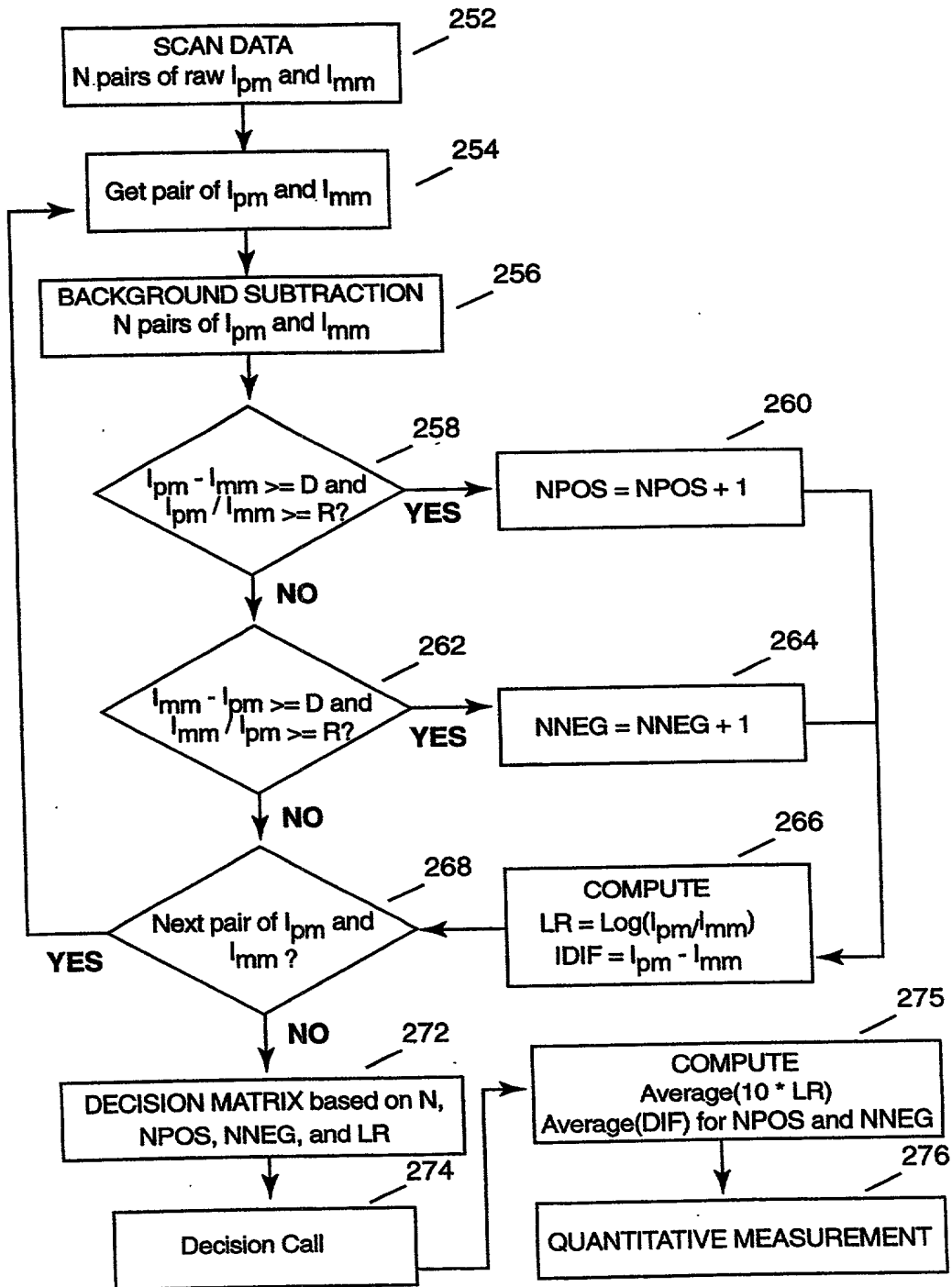


Figure 9

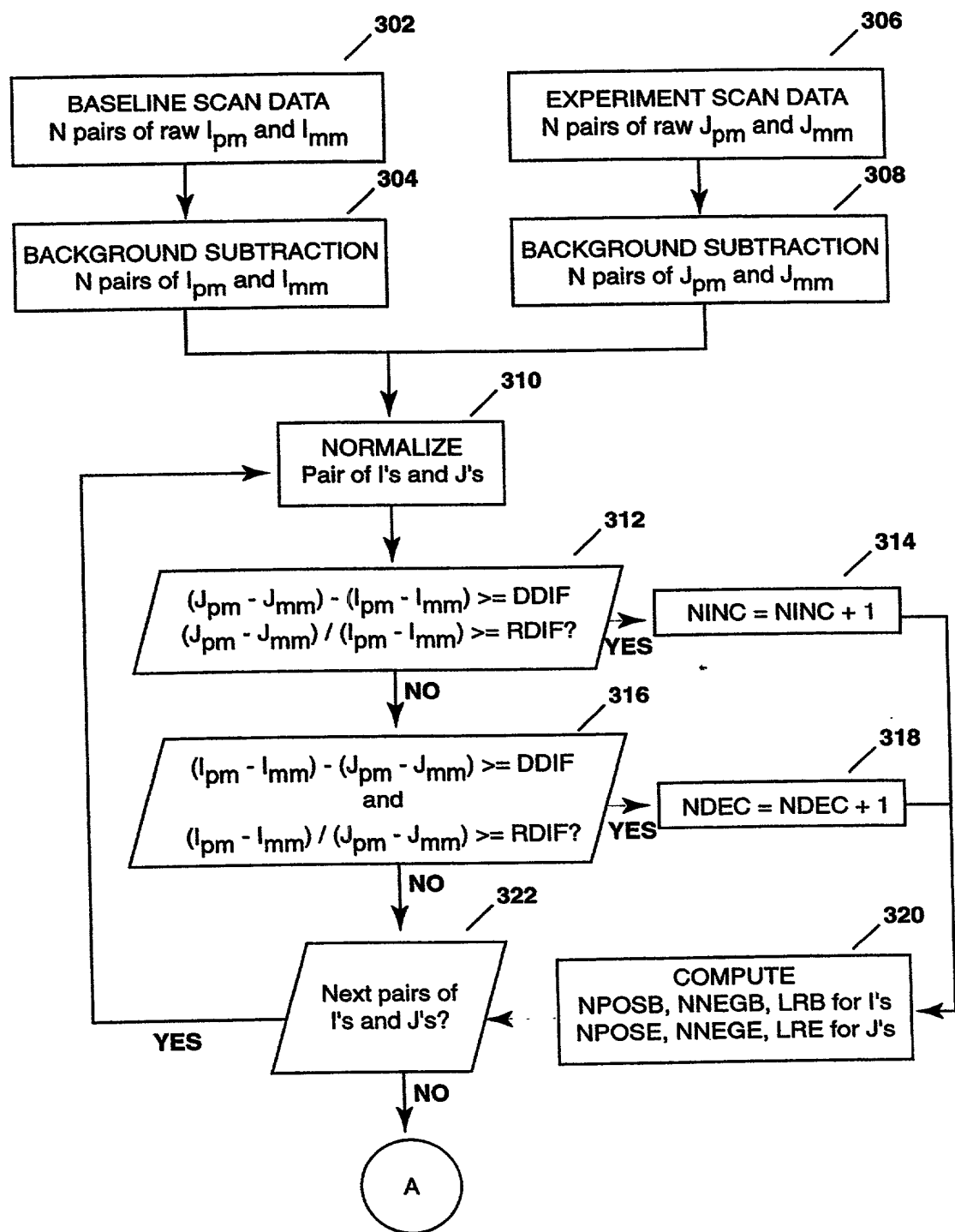


Figure 10a

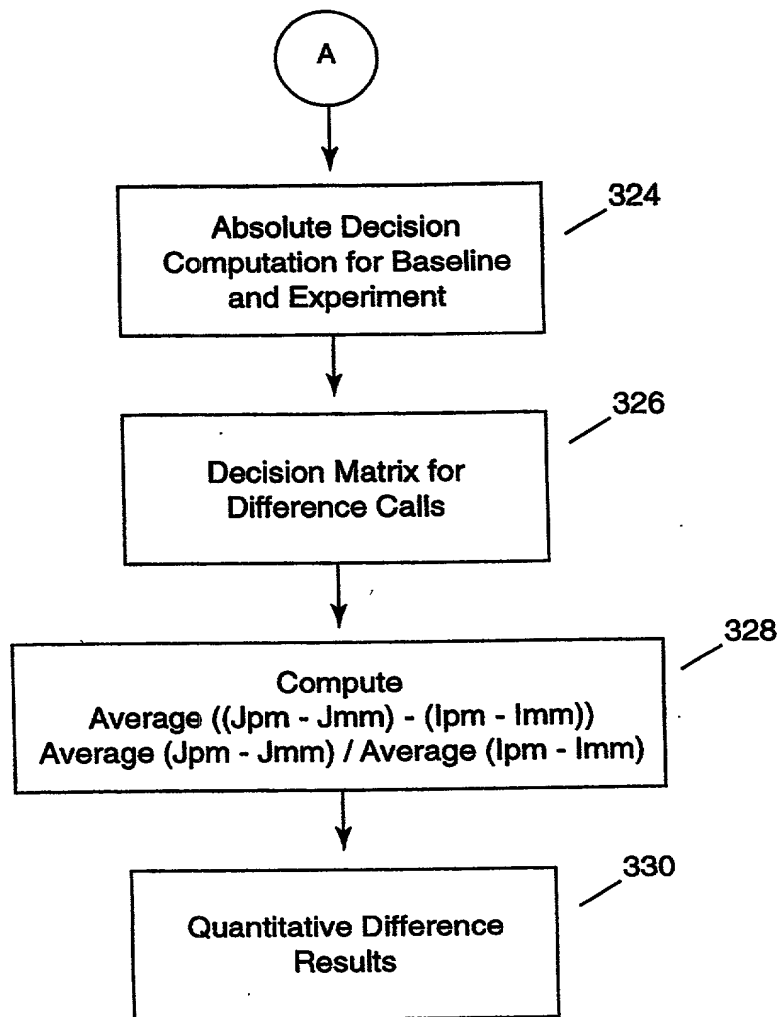


Figure 10b

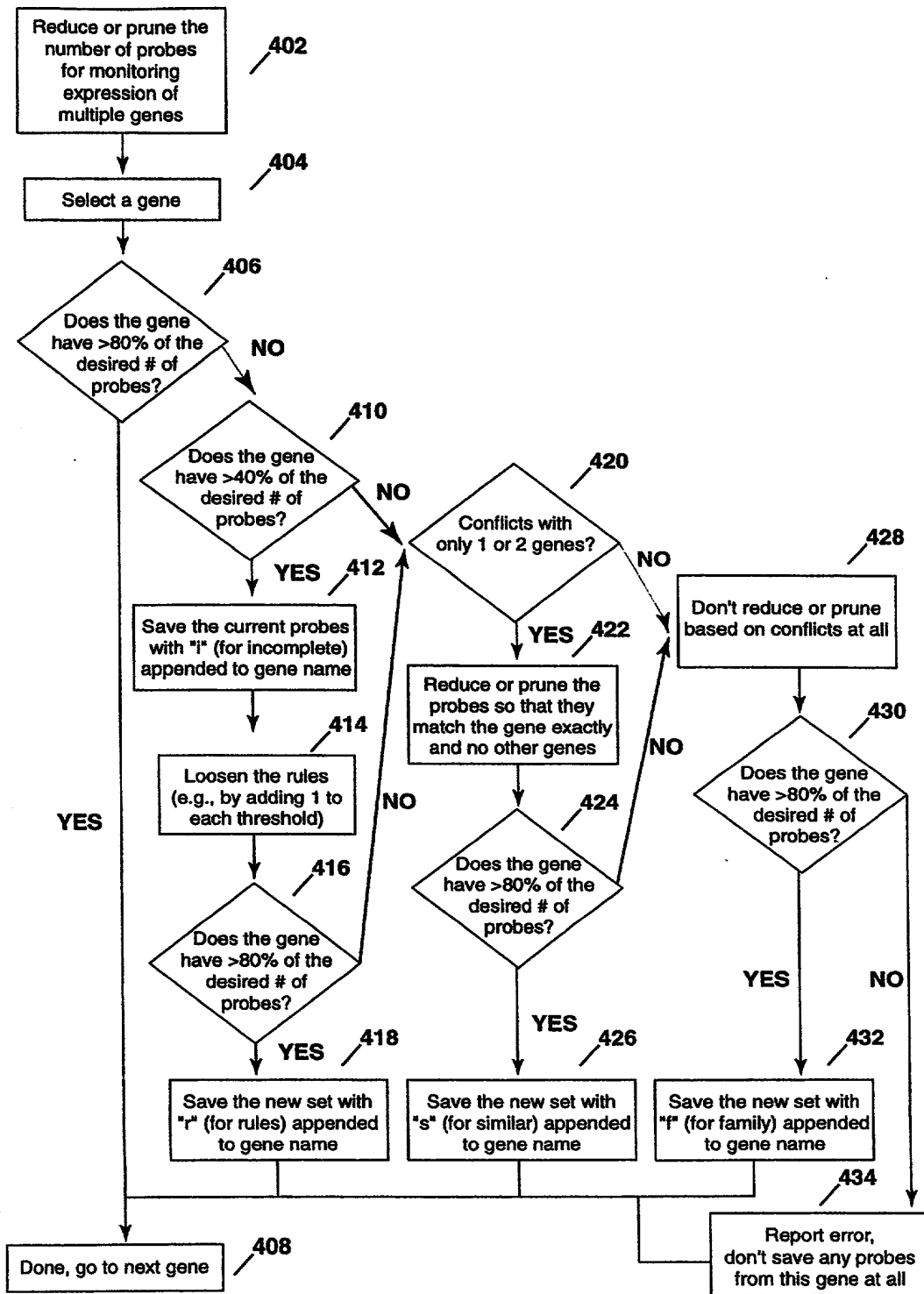


Figure 11

Discrimination with Ligation

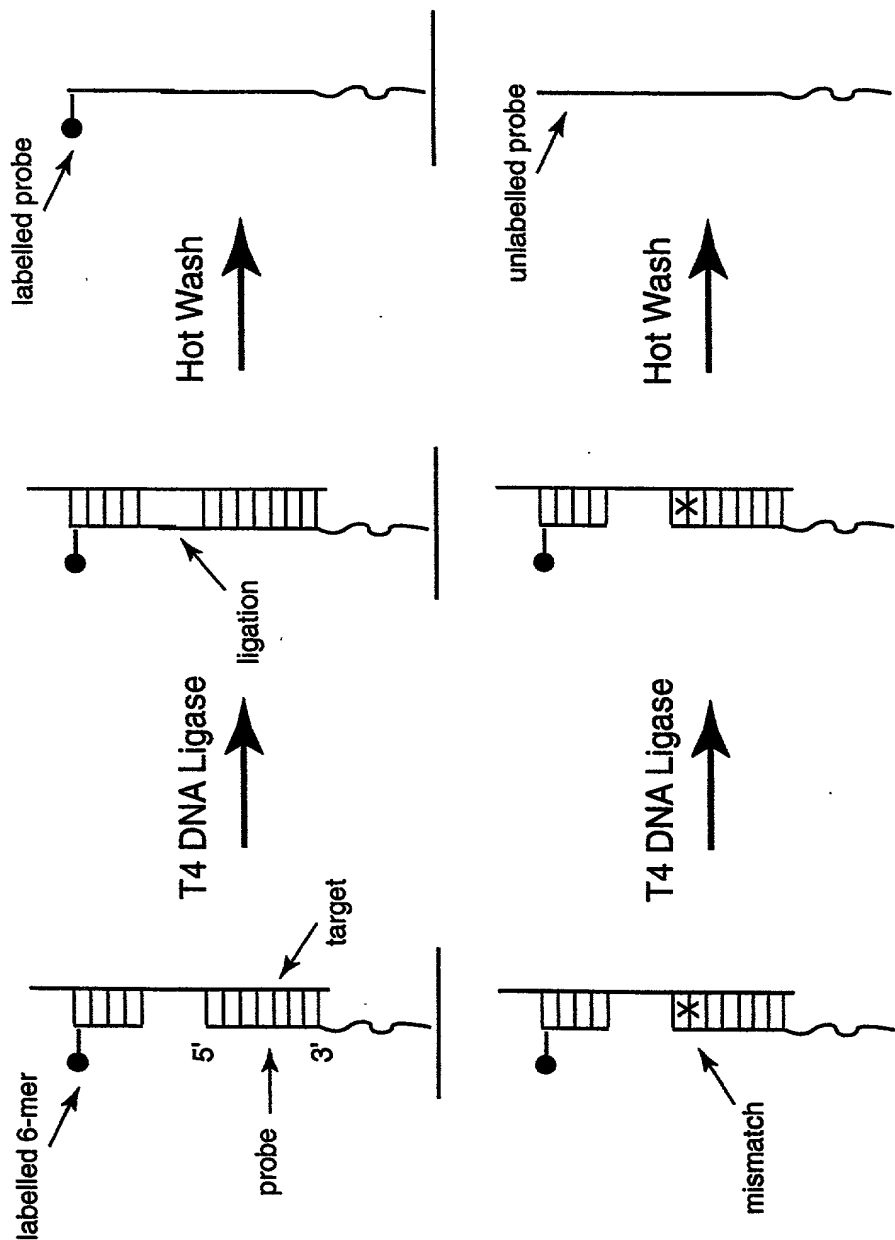


Figure 12

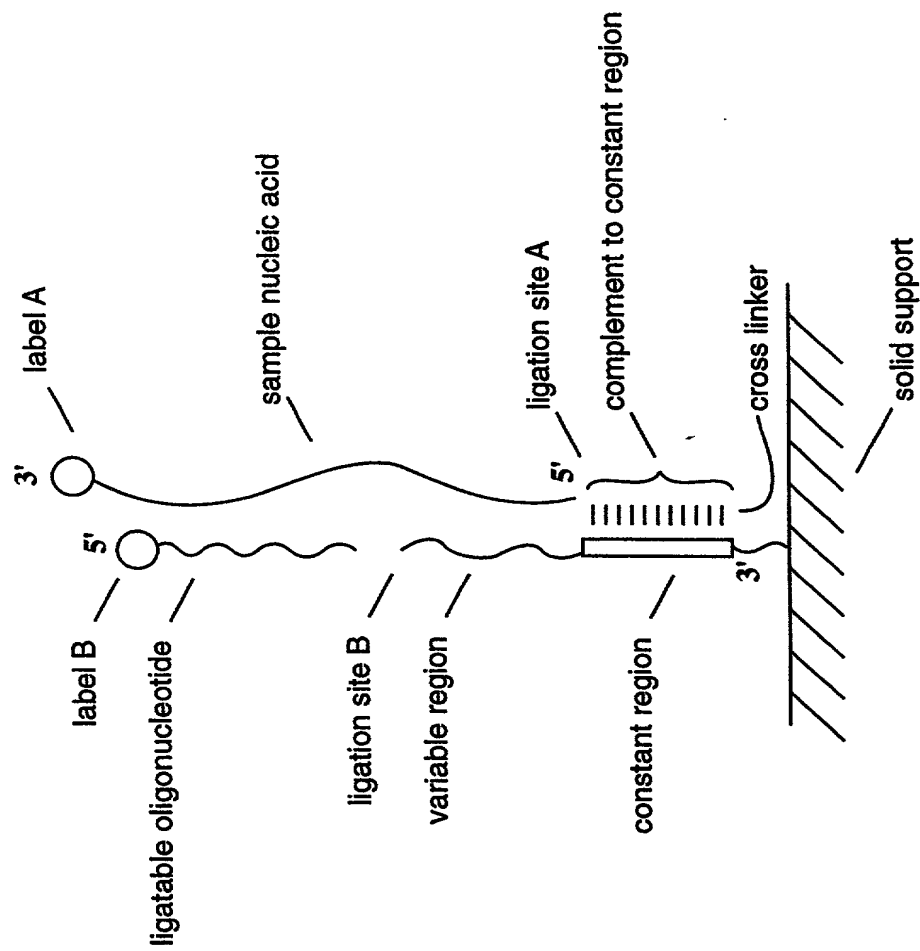


Figure 13a

Figure 13b

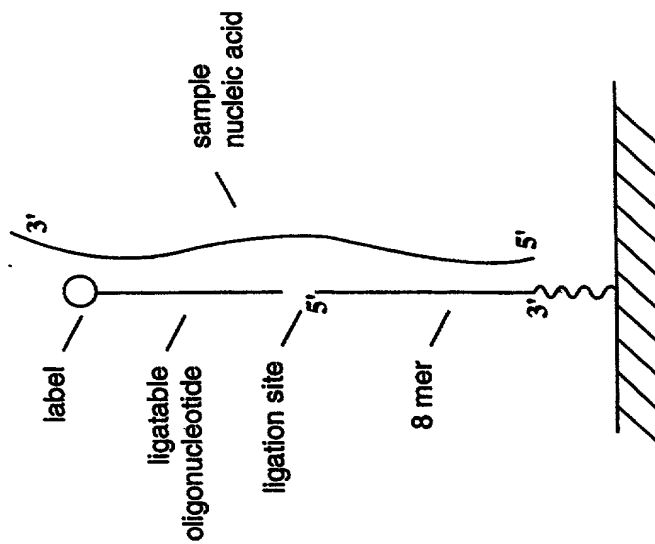
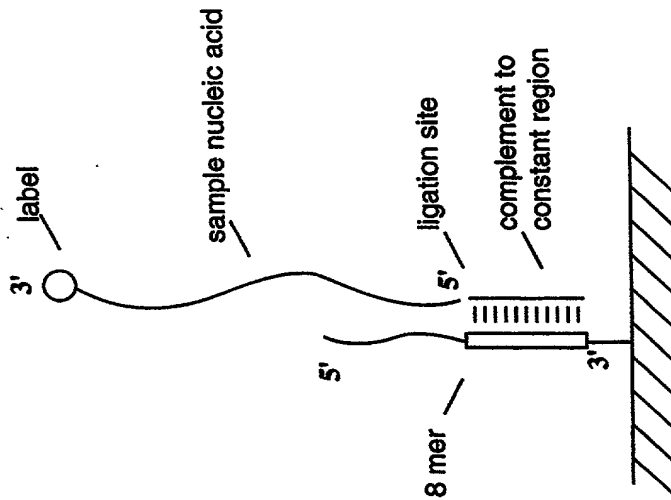


Figure 13c



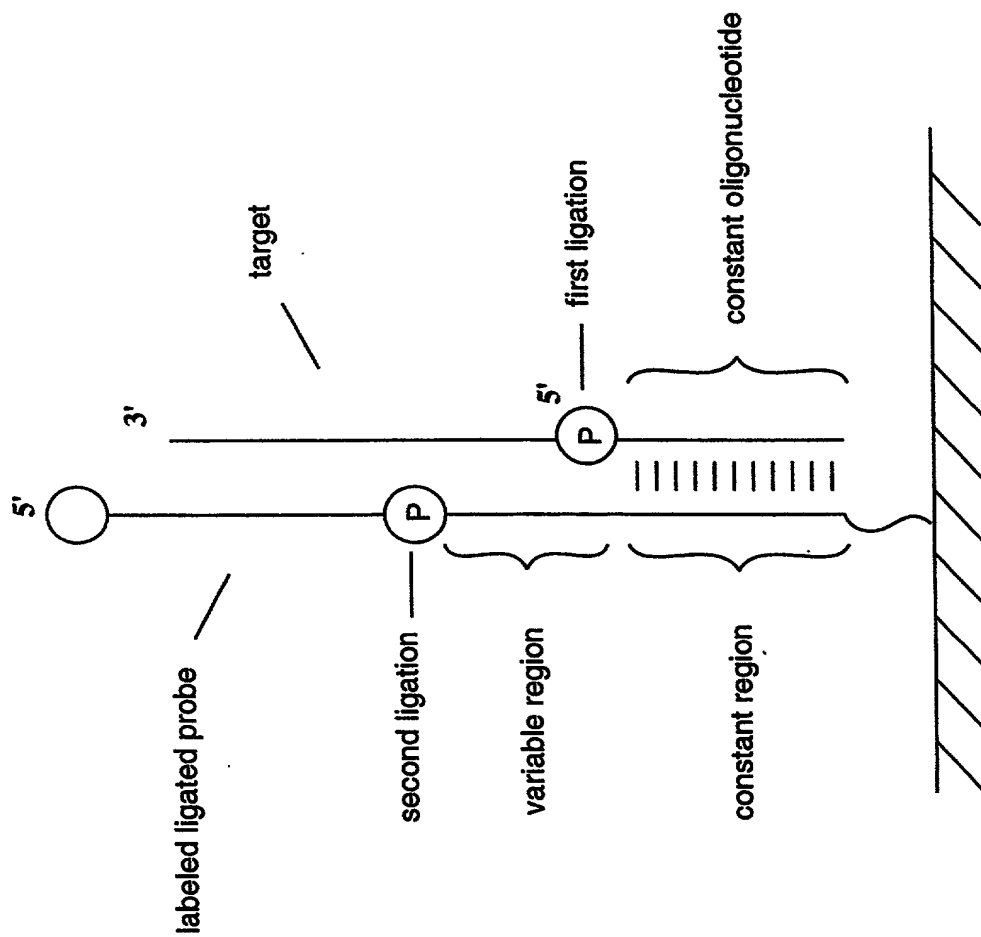


Figure 13d

Figure 14a

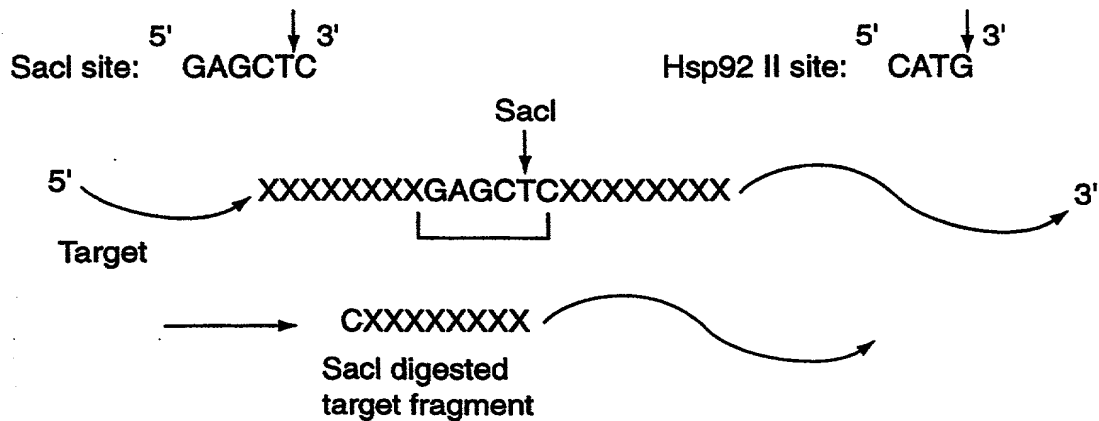


Figure 14b

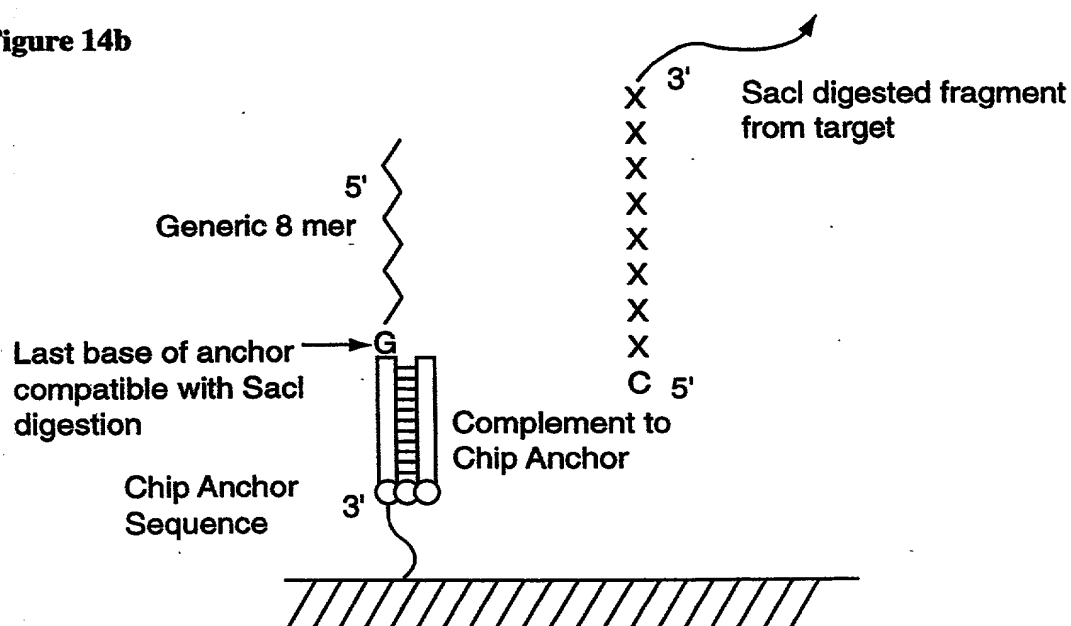


Figure 14c

Monitoring mRNA expression from organisms with small genomes:

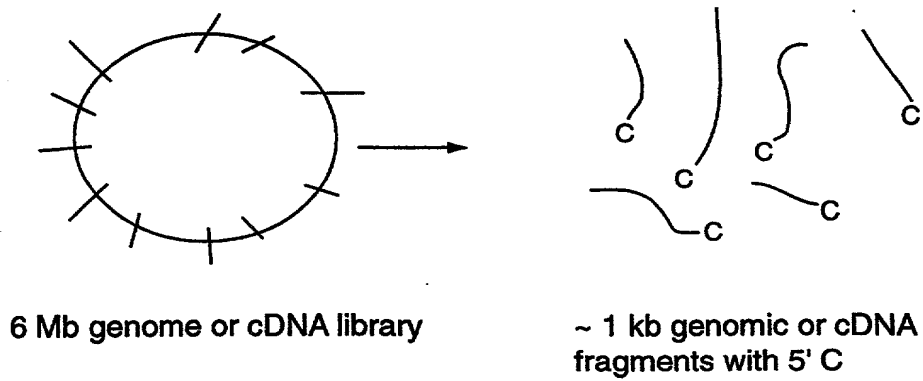
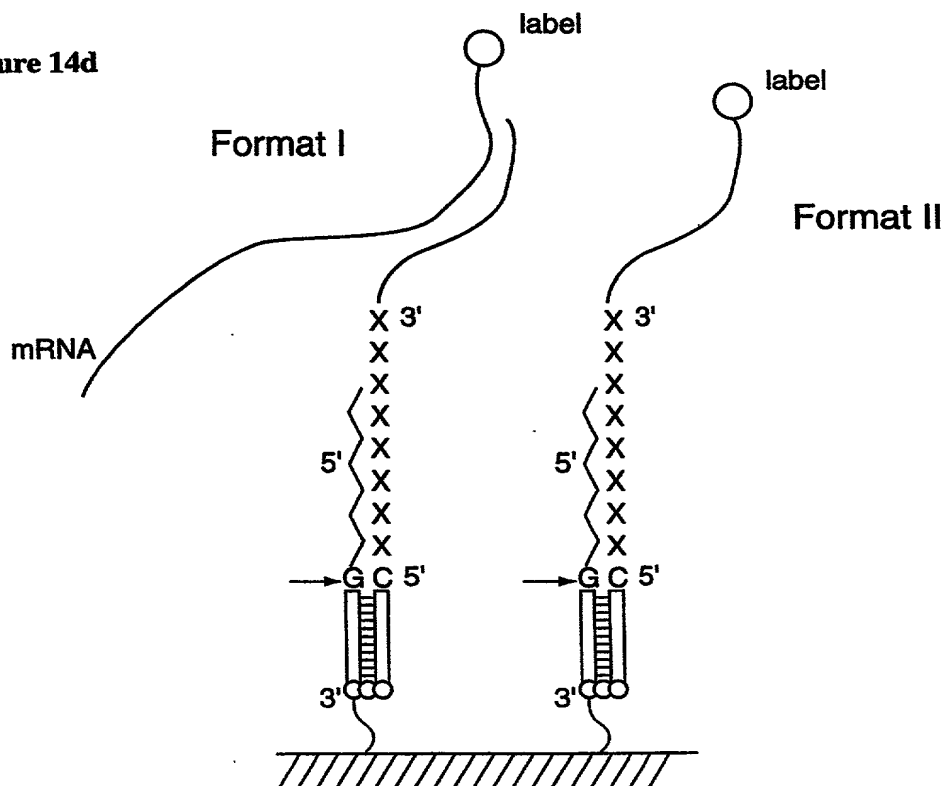
**Figure 14d**

Figure 15a

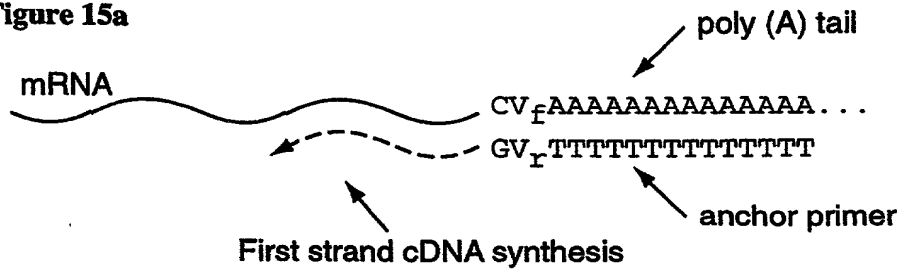


Figure 15b

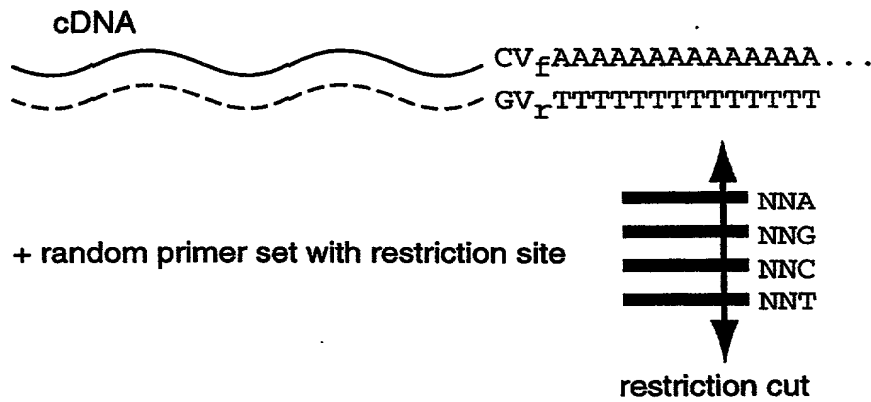


Figure 15c

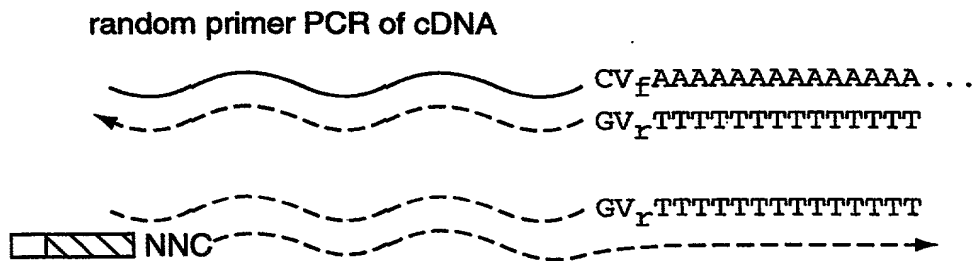
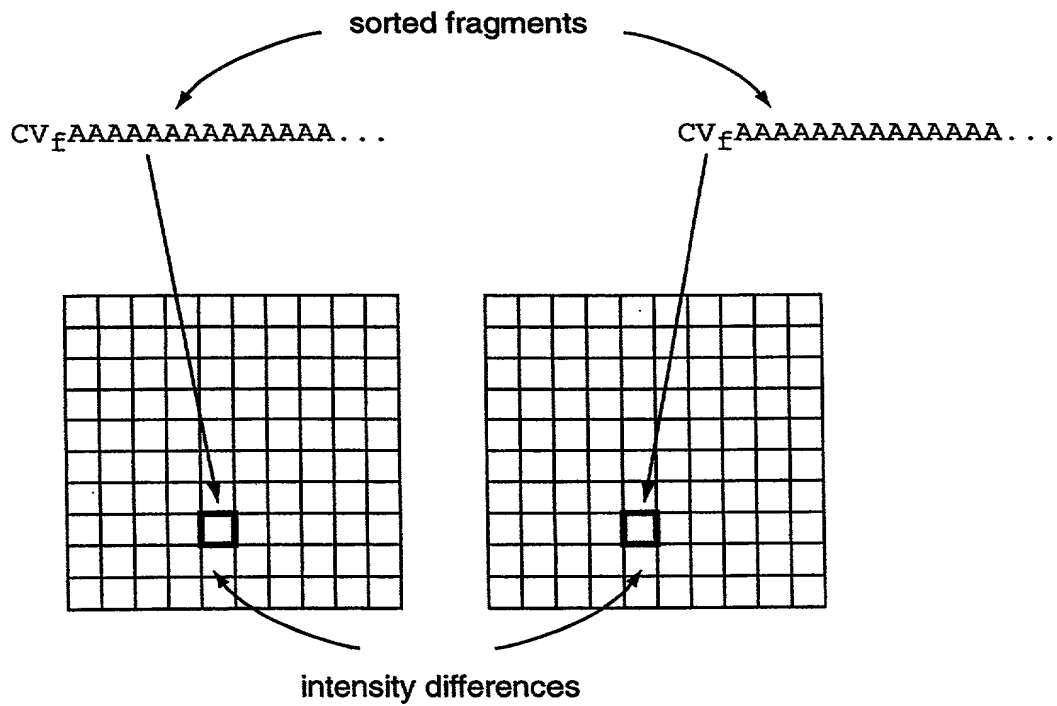


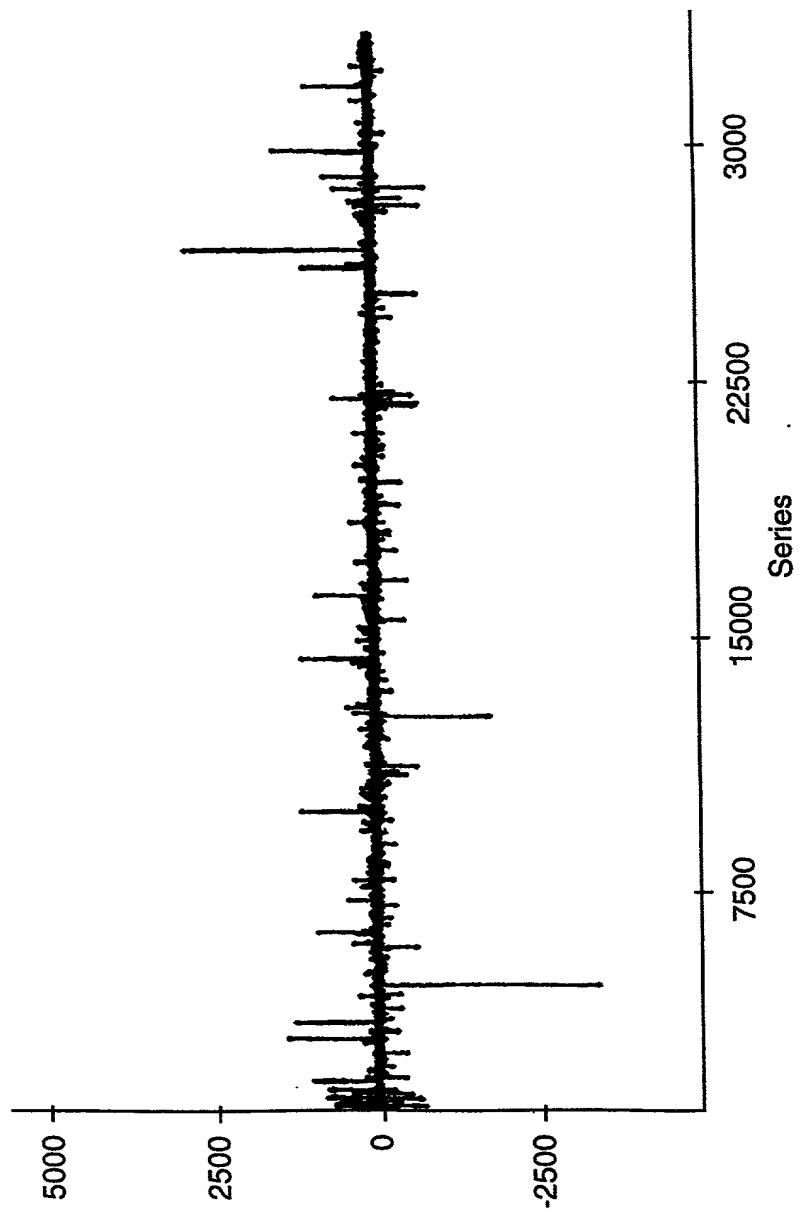
Figure 15d

Restriction digest PCR products

**Figure 15e**

Sort fragments by 5' ends on Generic Ligation GeneChip





Sample 1 vs. Sample 1 - Absolute Differences
(Replicate 1 vs. Replicate 2)

Figure 16a

400430 220350

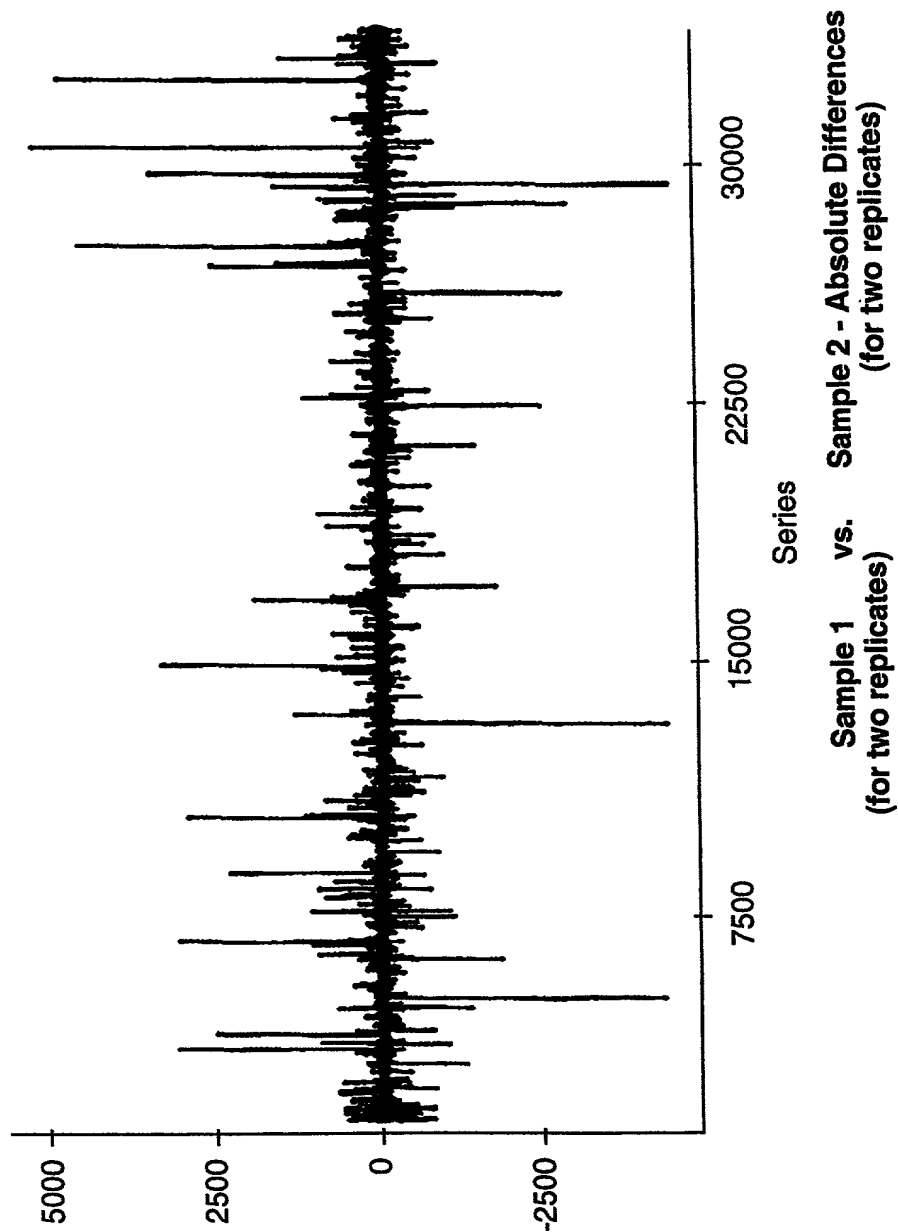


Figure 16c

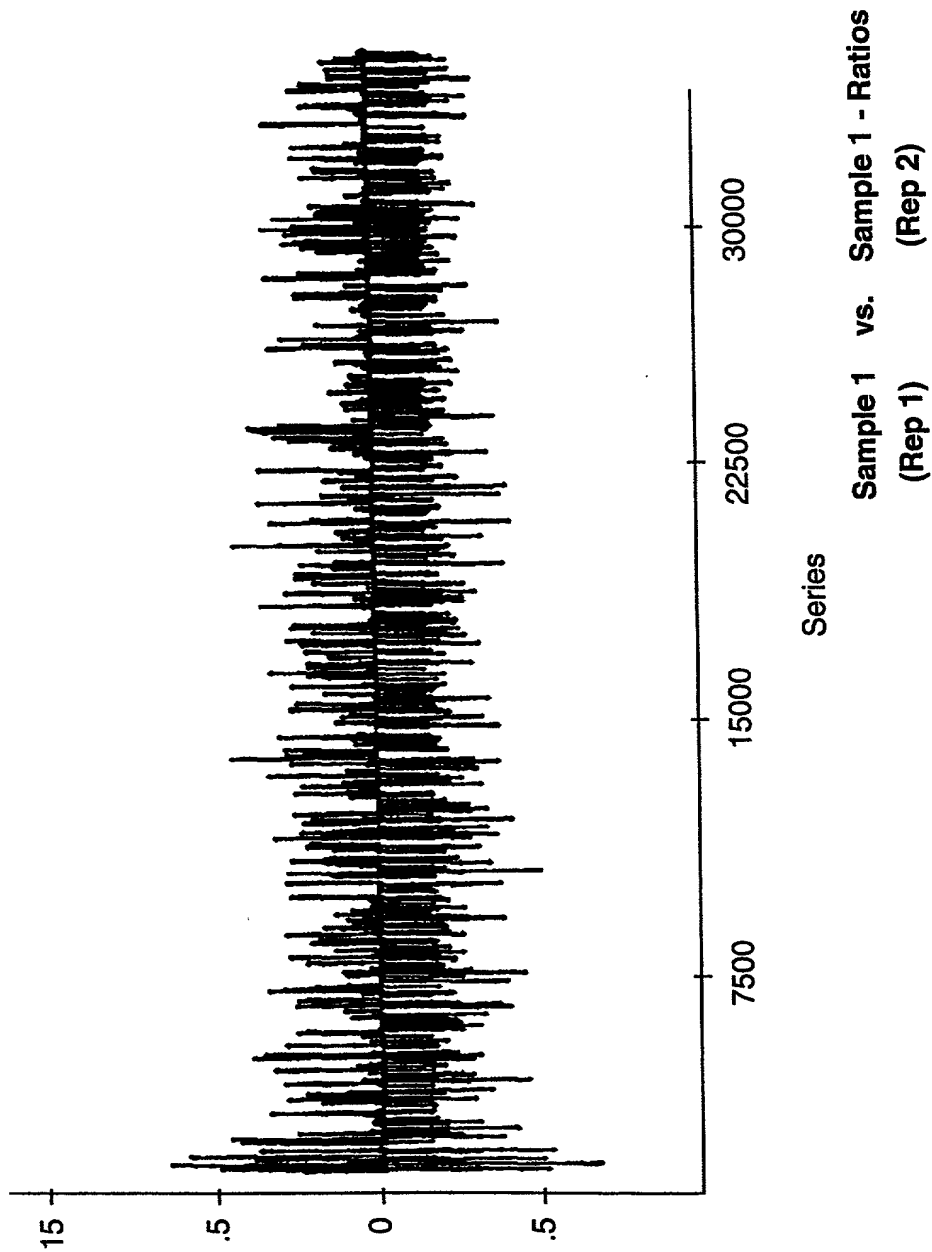


Figure 17a

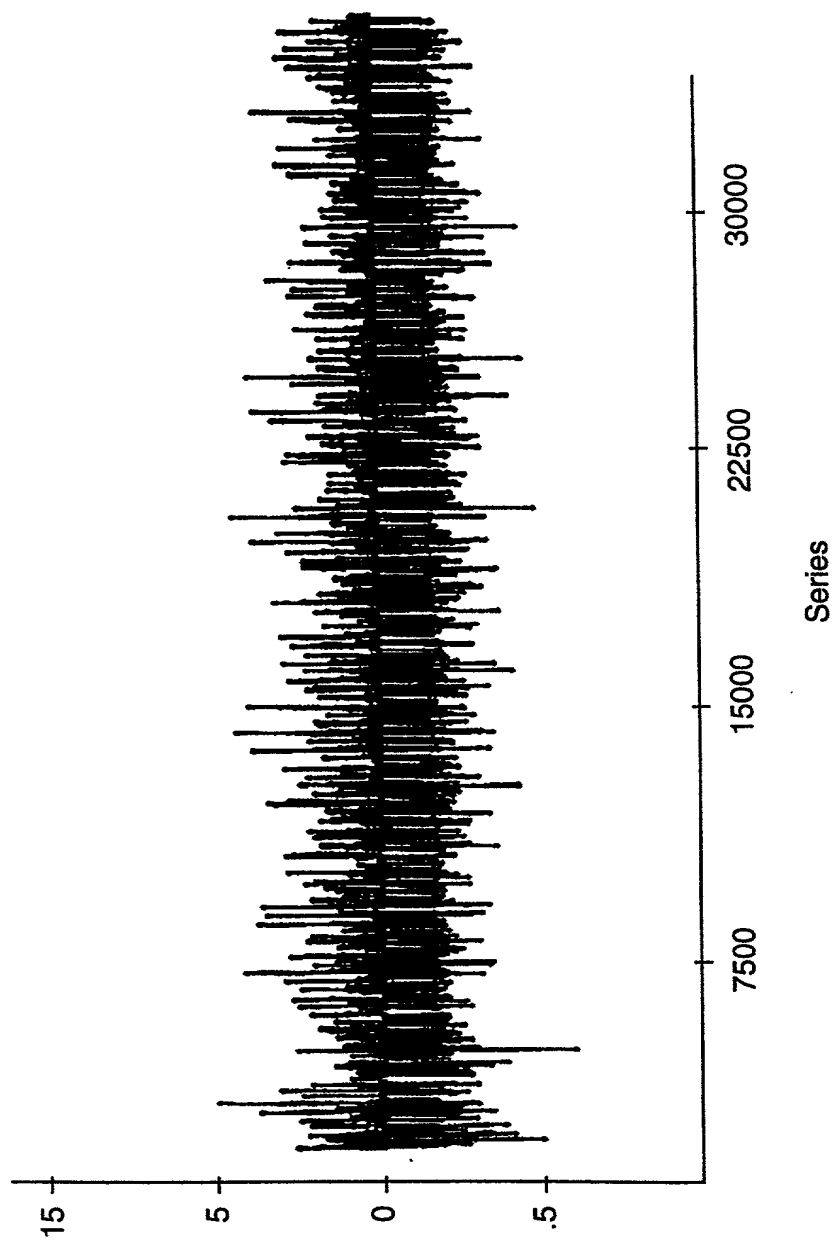
[illegible]

Figure 17b

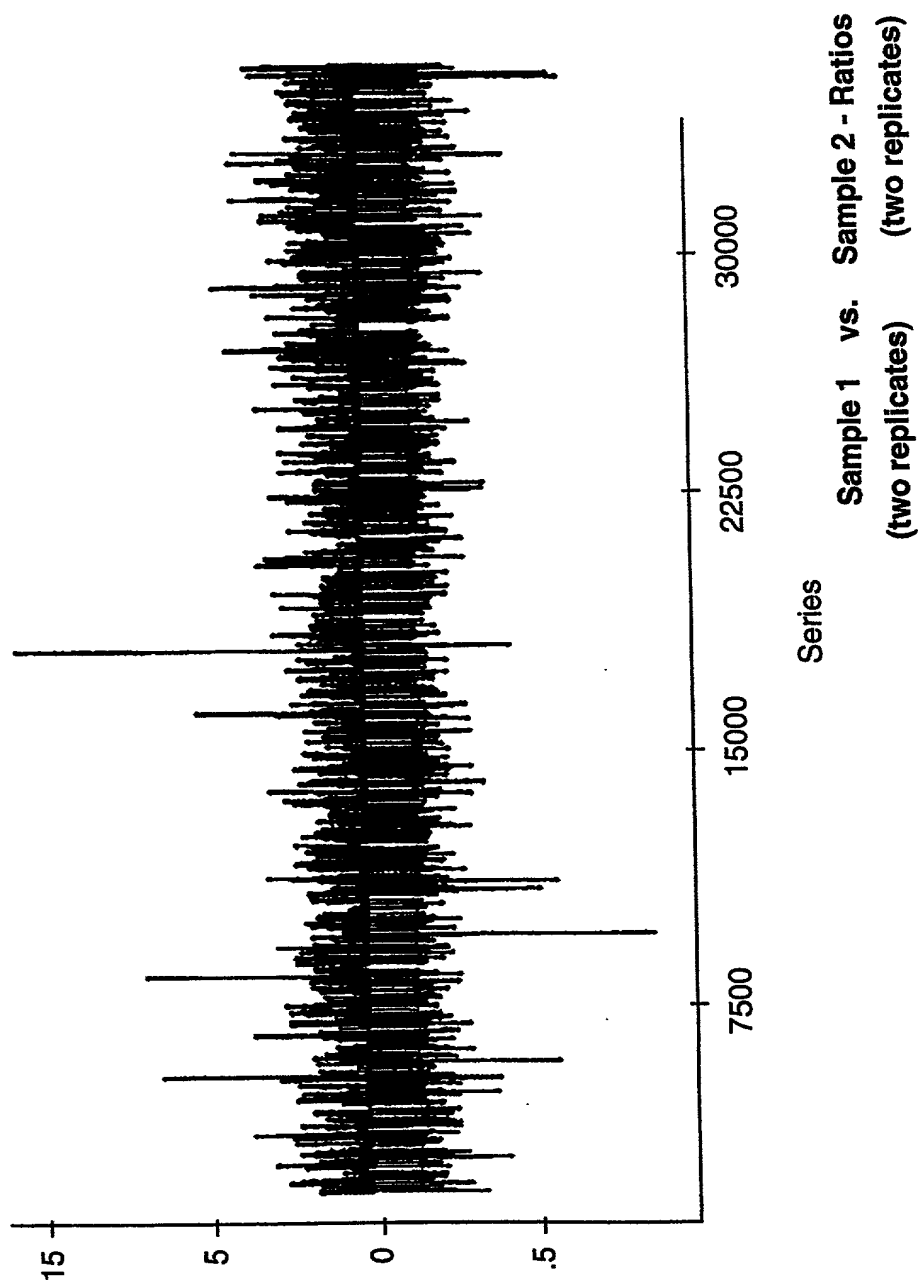
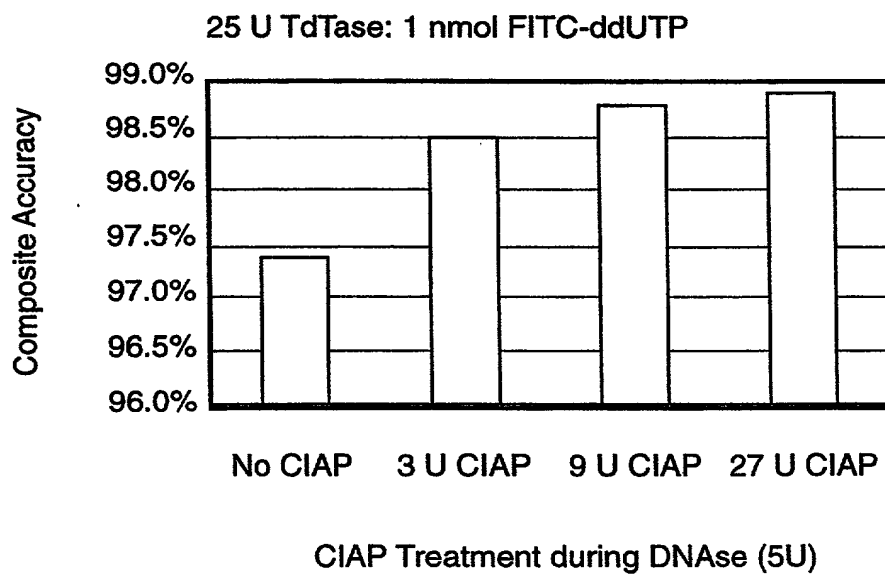
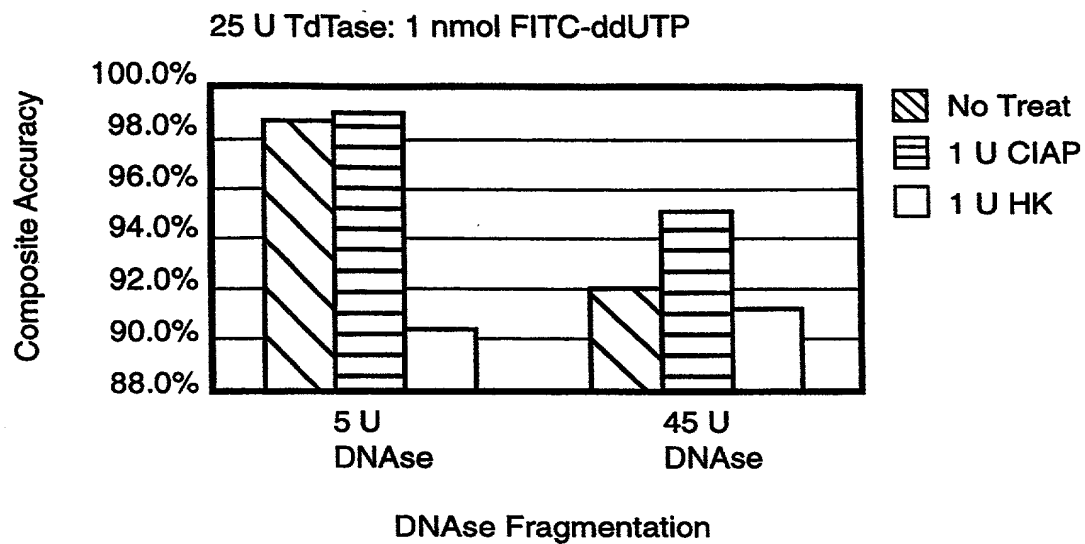


Figure 17c

Post-Fragmentation End Labeling: CIAP Treatment**Figure 18**

Post-Hybridization End Labeling on the Chip

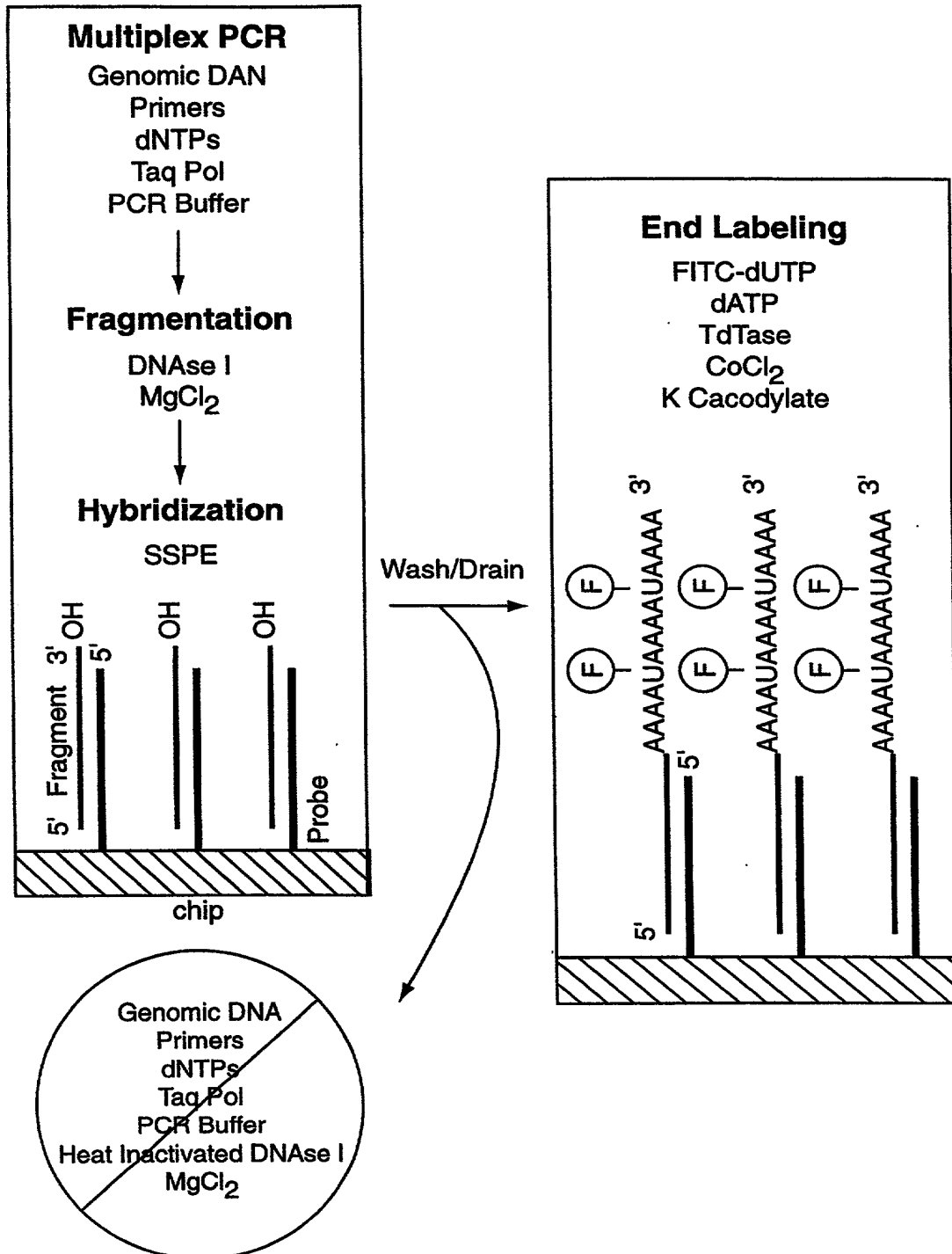


Figure 19

The diagram illustrates a three-step process for multiplexed PCR:

- Pre-React:** A chip with four DNA sequences (TTTTddT, TddT, TTddT, TTTTddT) is reacted with ddTTP, dTTP, TdTase, CoCl₂, and K Cacodylate. The 3' ends of the sequences are labeled with OH groups, and a probe is added.
- Fragmentation:** The chip is washed/drain, and the DNA sequences are fragmented. The 3' ends of the sequences are labeled with OH groups, and the probe is added.
- End Labeling:** The chip is washed/drain, and the DNA sequences are labeled with fluorescent dyes (F) at the 3' ends. The sequences are TTTTddT, TddT, TTddT, and TTTTddT.

Figure 20

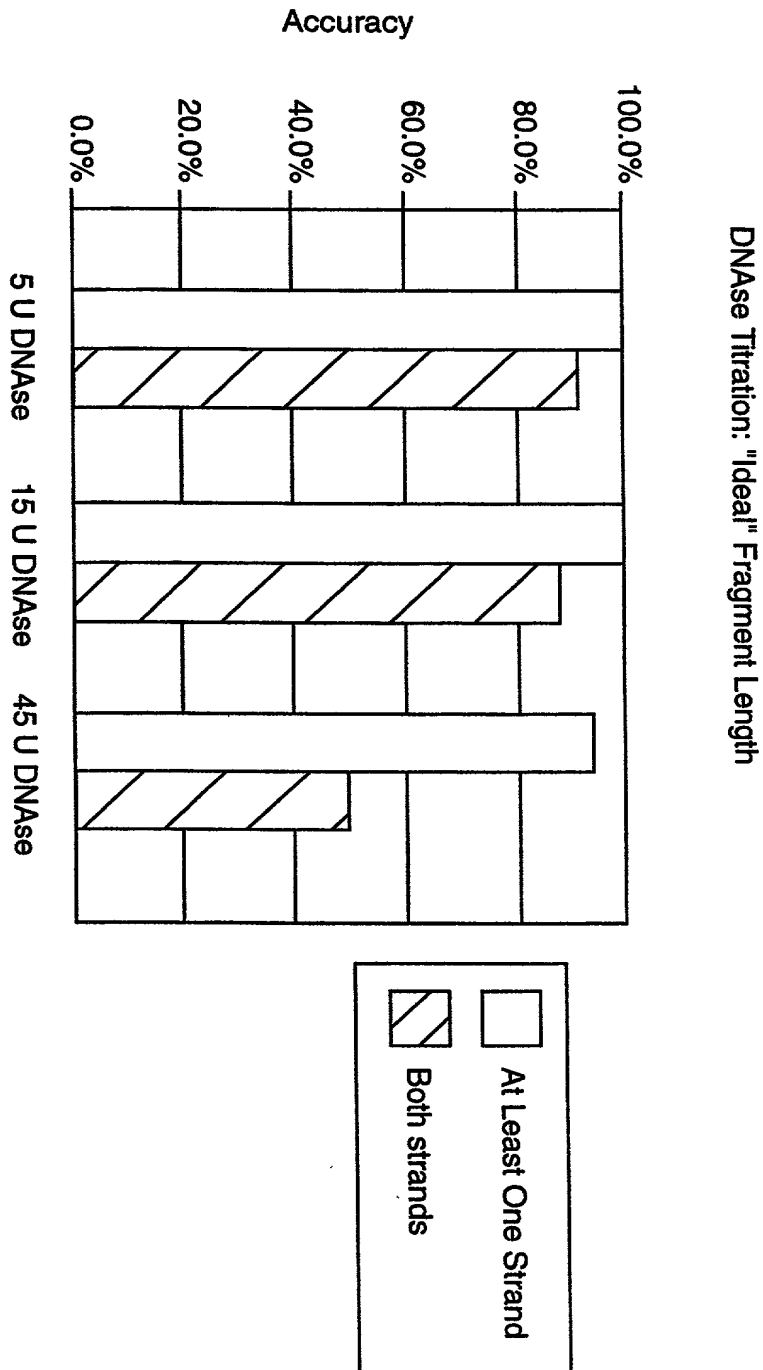
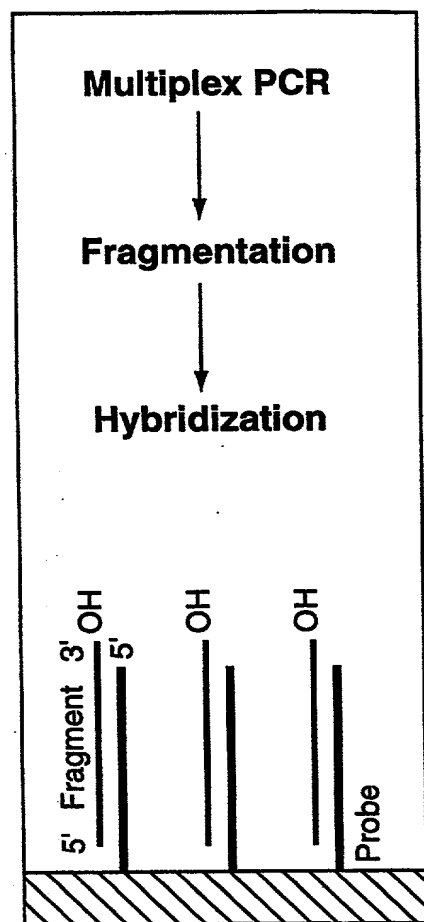


Figure 21

2020-07-27 10:23:41

Oligo dT Labeling on the Chip



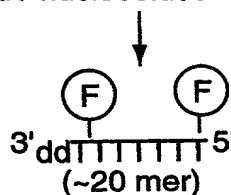
chip

Substitute FITC with:

- Rhodamine R110
- Cy fluorochrome

Oligo Synthesis

FITC-Phosphoramidite
ddT nucleoside at 3' end
dT nucleosides



Wash/Drain

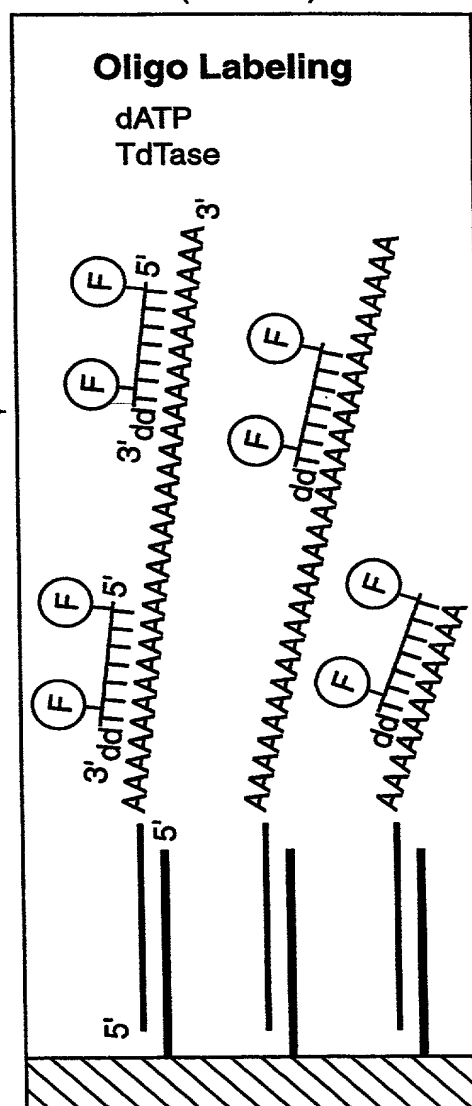


Figure 22

Labeling Reagents

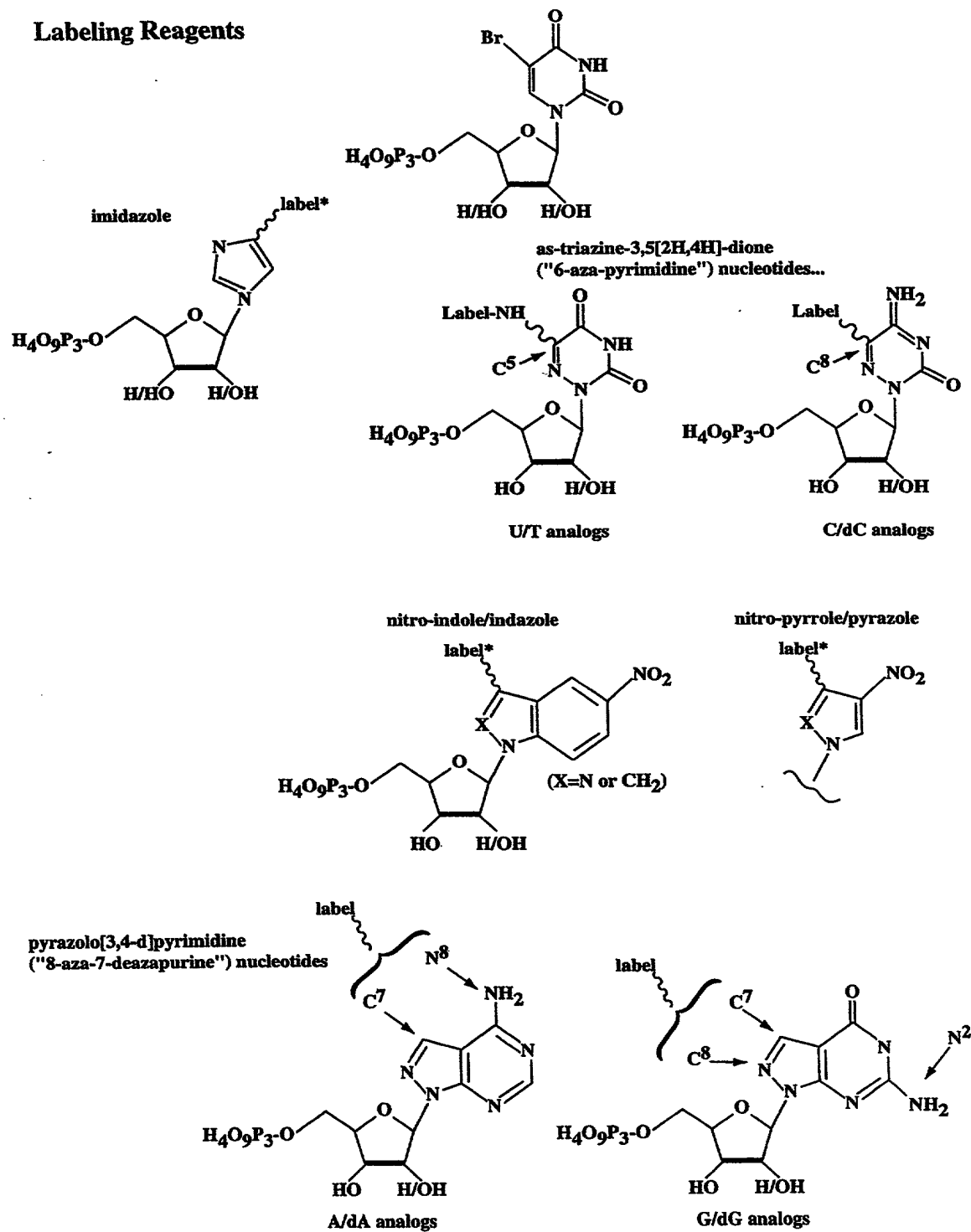


Figure 23a

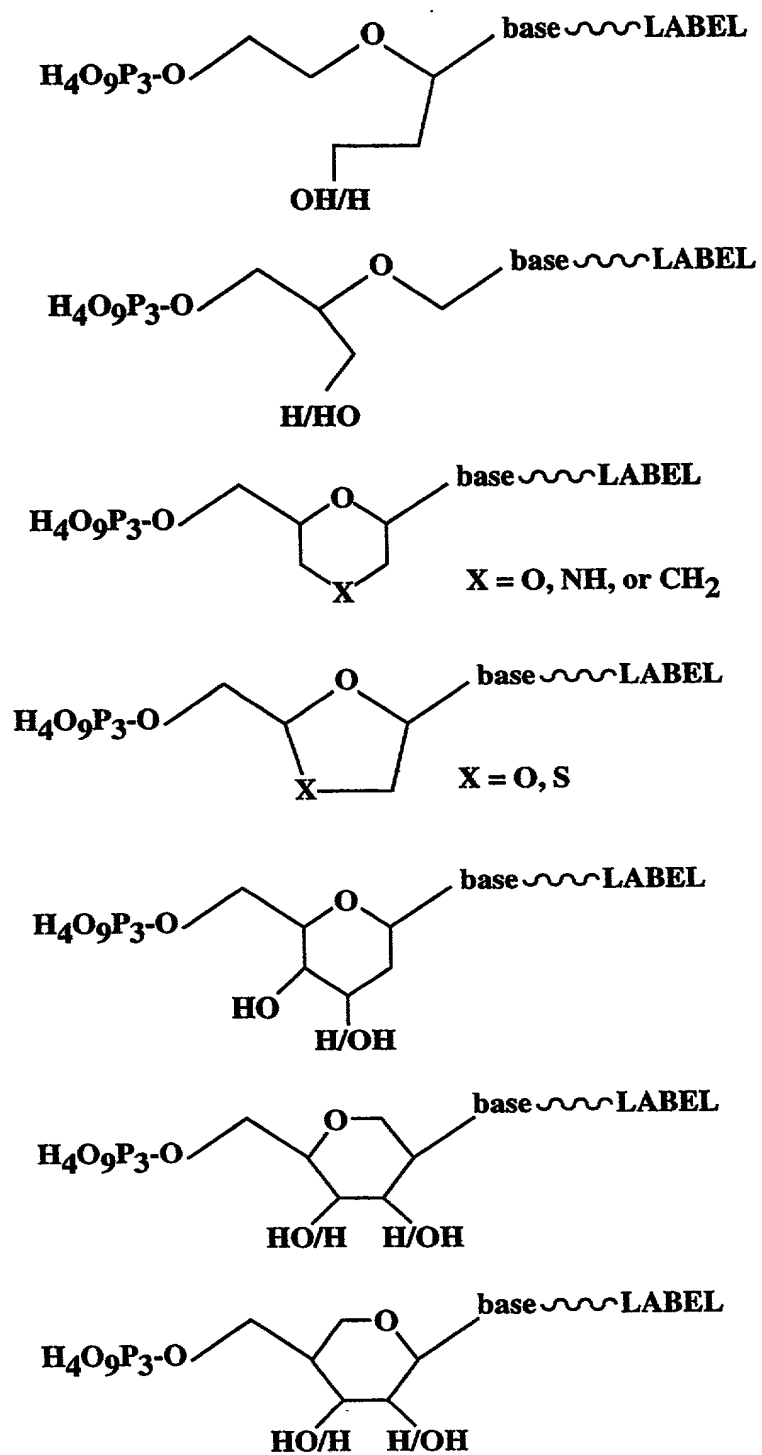
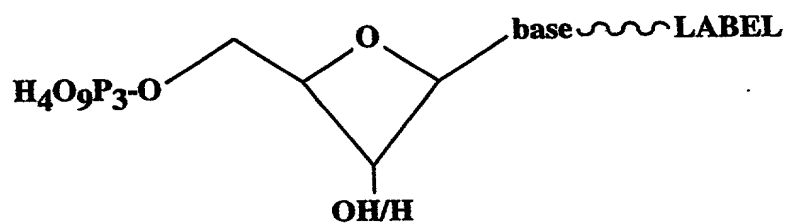
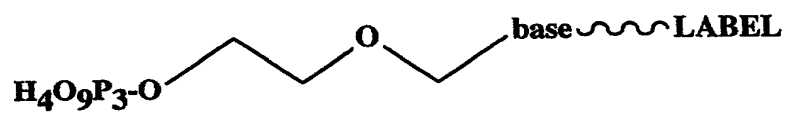


Figure 23b



base = heterocyclic moiety (eg. analogs thereof)
~~~~~ = linker;
LABEL = detectable signal-gene

Figure 23c

Resequencing a target DNA molecule with a set of generic n-mer tiling probes

ie. 4-mer probes:

Target: 5' TGACATAGGACAGCGAAGGGA... 3'

Probe 1: ACTG 5'

Probe 2: CTGT

Probe 3: TGTA

GTAT

Probe 5: TATC

ATCC

TCCT

CCTG

Probe 9: CTGT...etc.

Figure 24

Four electronic tiling arrays are present on a 4-mer generic array:

(4 x 3 = 12 "nearest neighbors" for each probe)

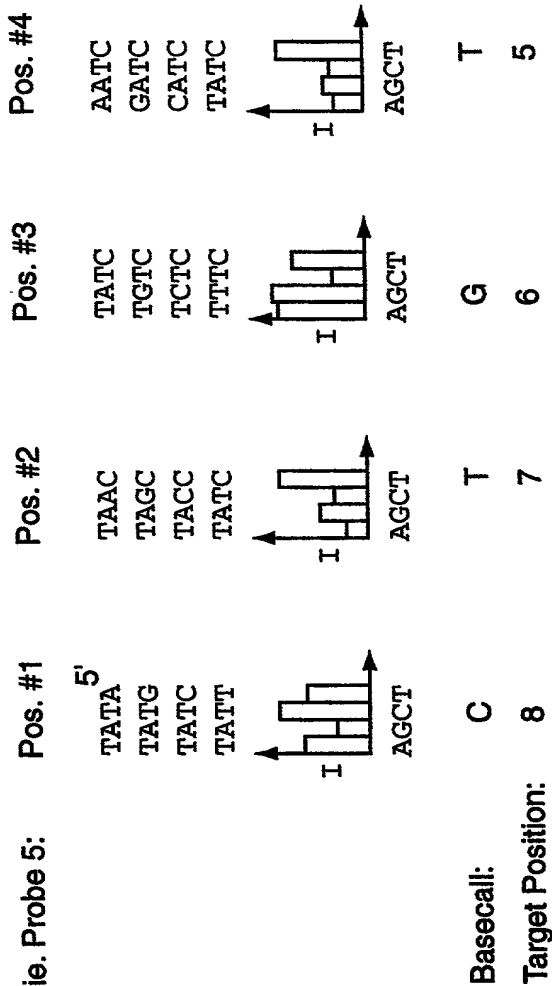


Figure 25

Base Vote Table

Base Position	Base Identity	Base Vote	Correctness Score Total	Correctness Score #1	Correctness Score #2	Correctness Score #3	Correctness Score #4
5	T	T	1	1	0	1	0
6	A	A	1	1	1	1	1
7	T	T	1	0	1	0	1
9	C	G	0	0	0	0	0
10	T	T	1	1	0	1	0
11	G	G	1	0	1	1	1
12	T	T	1	0	1	1	1
13	C	C	1	1	0	0	1
14	G	G	1	0	1	1	1
(10) TOTALS							

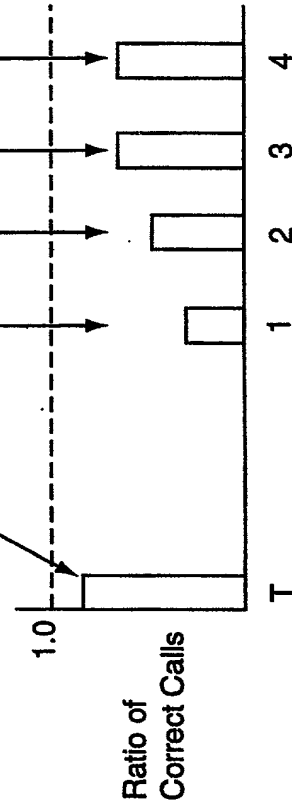
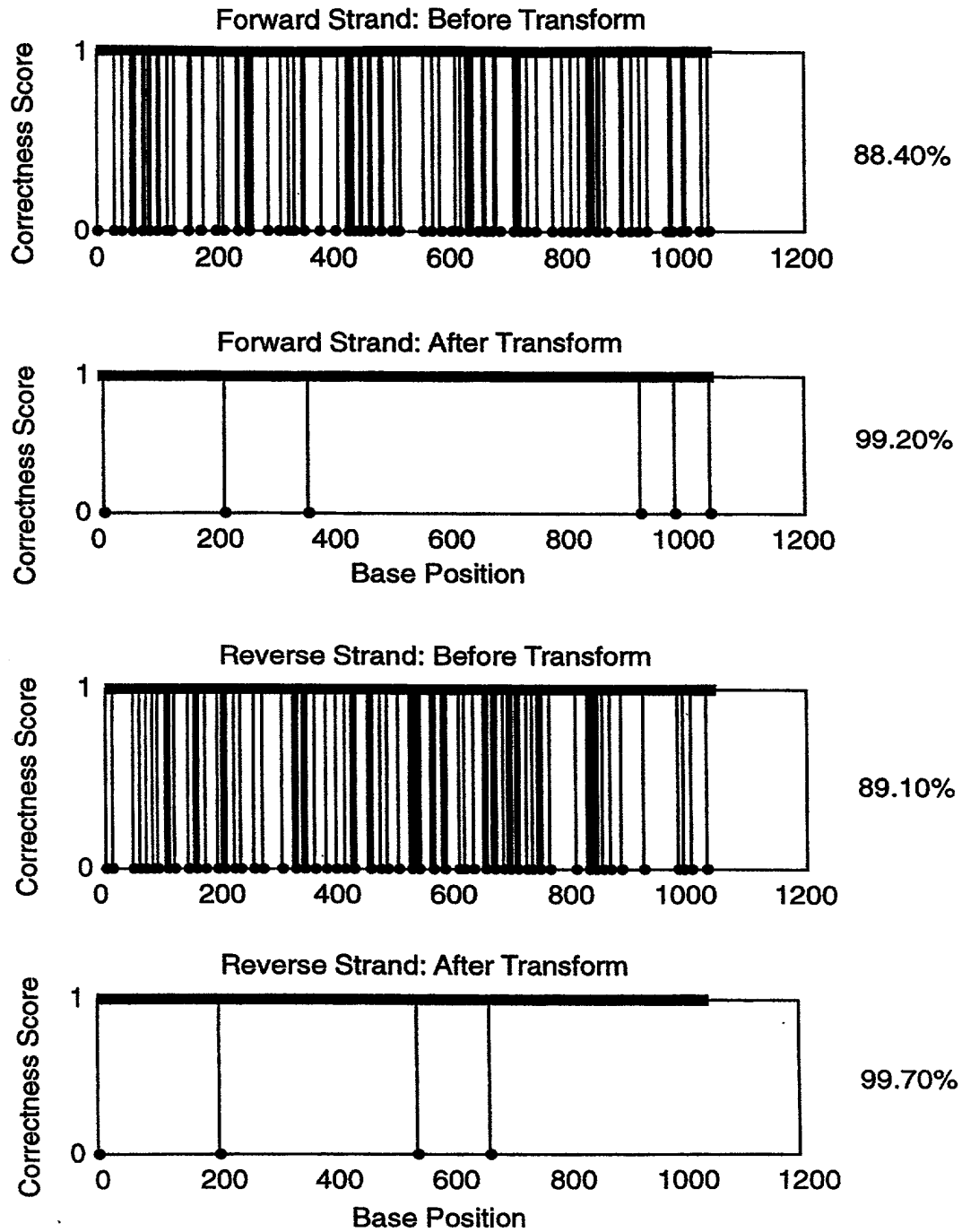
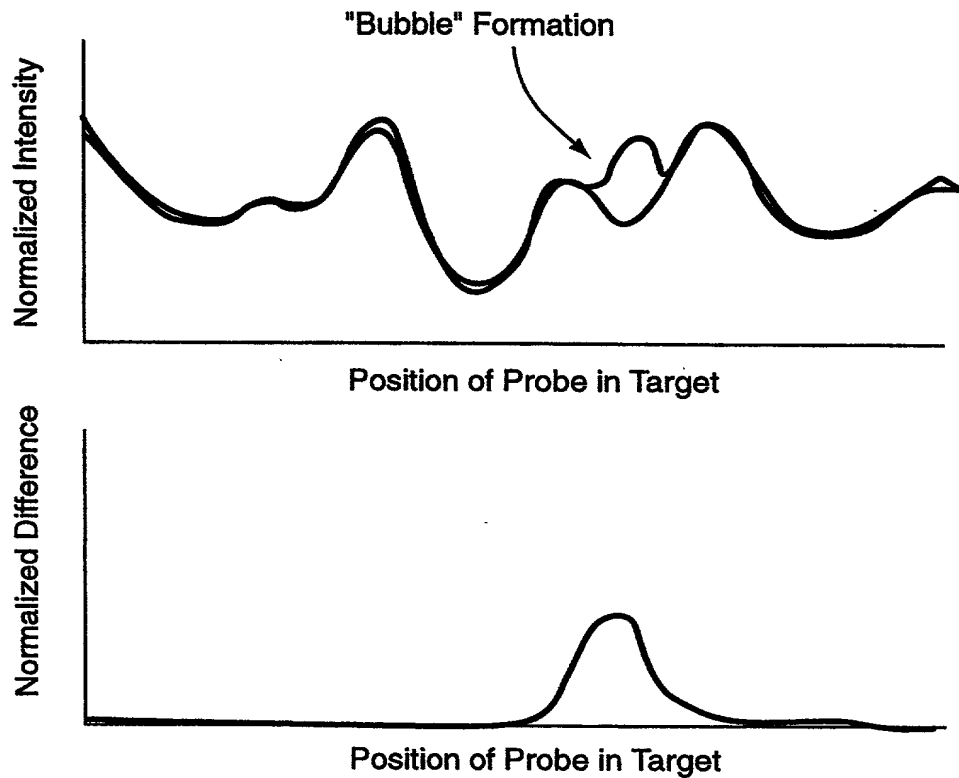


Figure 27

Effect of Applying Correctness Score Transform to HIV Data**Figure 28**

Mutation Detection by Intensity Comparisons



Algorithms:

$$I_{\text{normalized}} = I_{\text{probe}} / (\sum I_{\text{NN}})$$

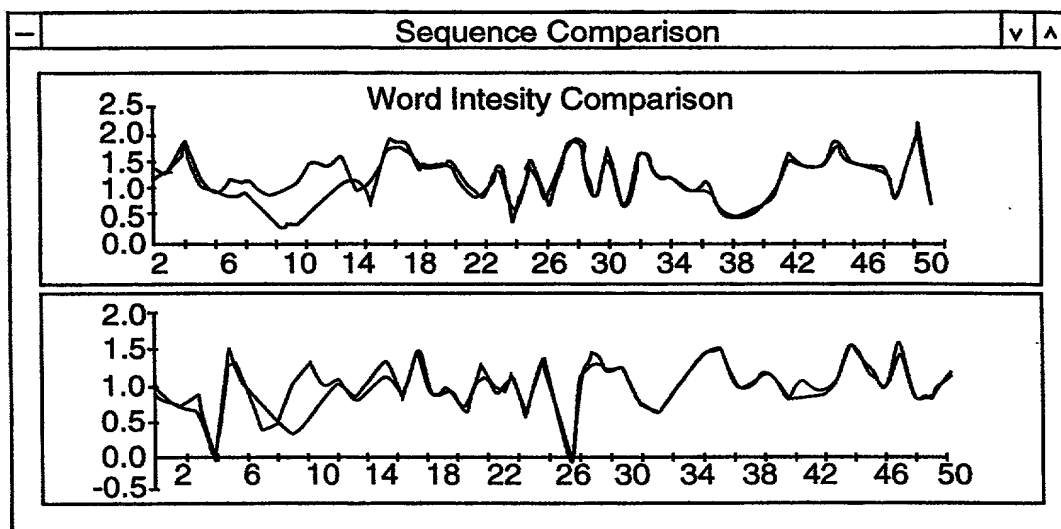
$$I_{\text{difference}} = \frac{(I_{\text{normalized, variant}} - I_{\text{normalized, control}})}{(I_{\text{normalized, variant}} + I_{\text{normalized, control}})}$$

- Locally normalized intensities track well
- Local normalization is sensitive to mutations

Figure 29

Bubble Formation Detection of Mutation in HIV Genome

Normalized Intensity Comparison:



Normalized Difference:

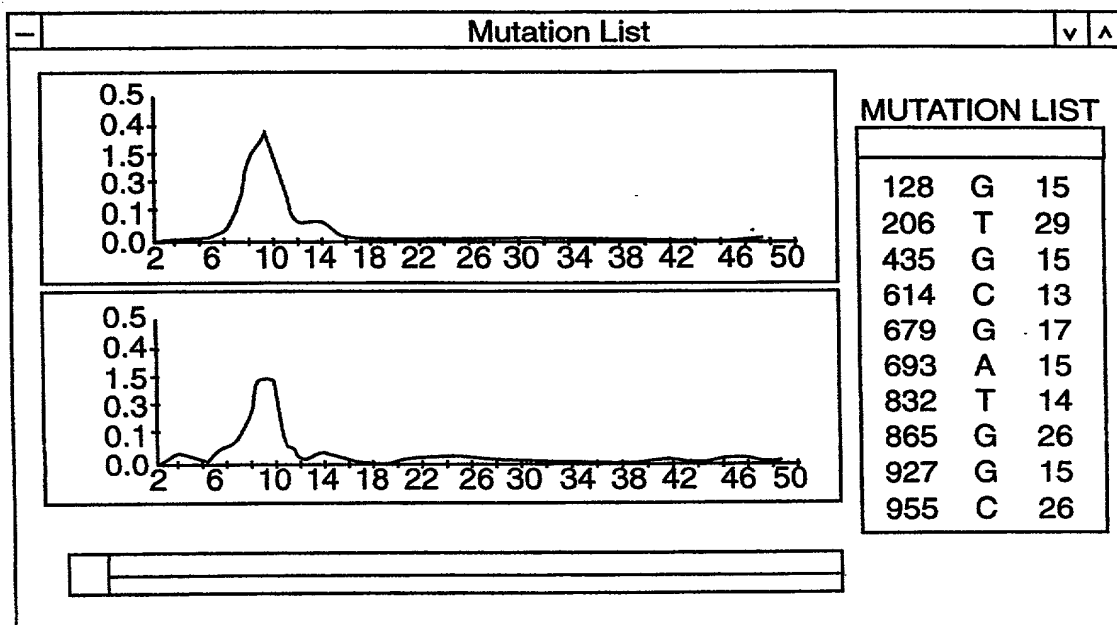
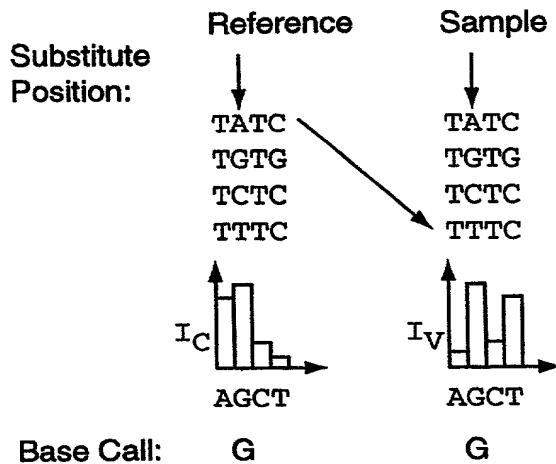


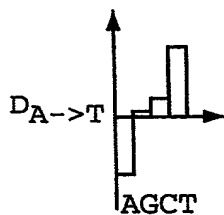
Figure 30

Induced Difference Nearest Neighbor Probe Scoring:



Induced Difference: $D_A = (I_{V,A} - I_{C,A}) / I_{C,A}$

- Average induced differences over all tilings and over both forward and reverse strands.



- Probe with A - "down-regulated"
- Probe with T - "up-regulated"
- A → T mutation

- Total Induced Difference > + Threshold: Mutation Exists
- Total Induced Difference < - Threshold: Mutation Exists
- Two criteria for mutations: Induced Difference Scores; Bubble Formation

Figure 31

Mutations found in an HIV PCR target (B) using a generic ligation GeneChip and induced difference analysis

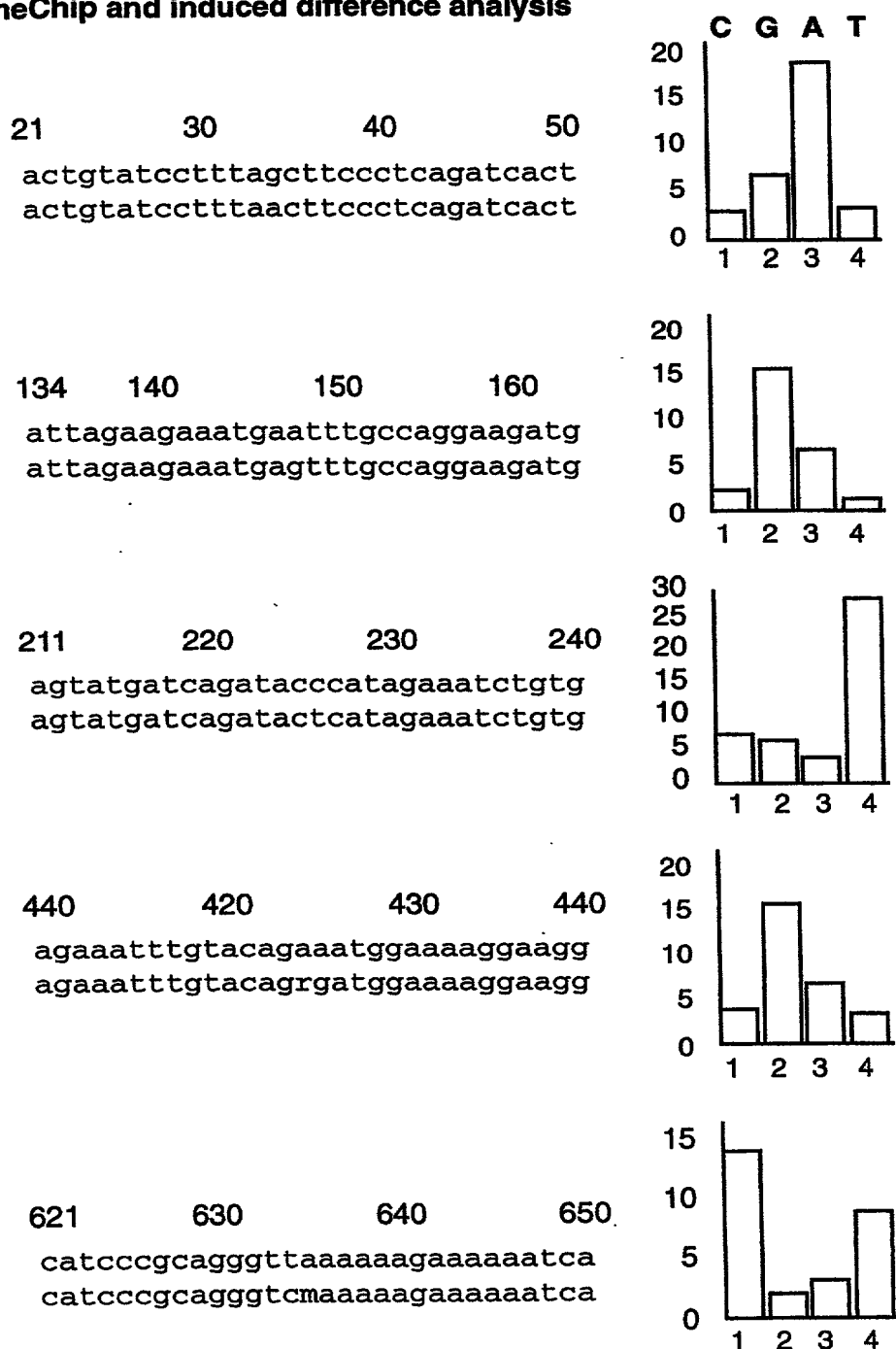


Figure 32a

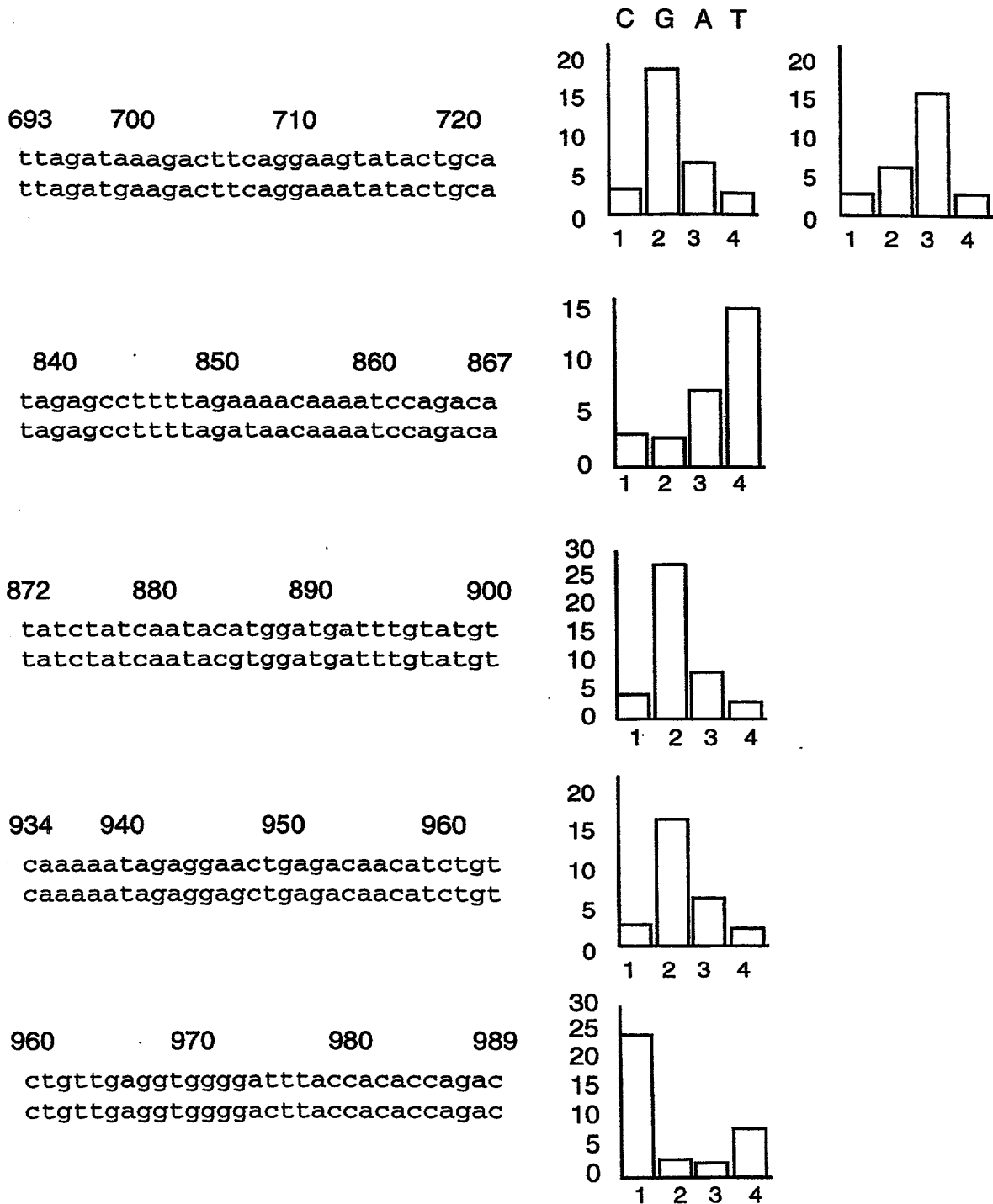
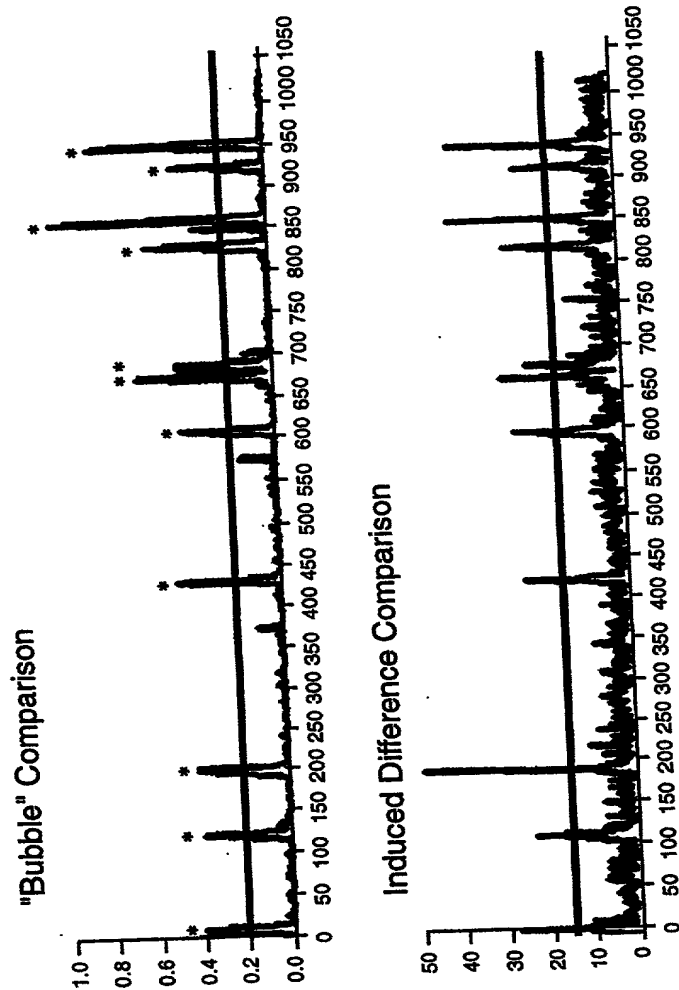


Figure 32b

Mutation Detection Using Comparisons Between a Reference Target and a Sample Target



Results: No false positives, all 11 mutations (indicated by *) are detected in this 1041 bp HIV DNA fragment.

Figure 33